University of Alberta

Glucagon-Like Peptide 2 and Dexamethasone Alter The Ontogeny of Lipid Absorption in Suckling and Weanling Rats

by

©

Claudiu Iordache

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment

of the requirements for the degree of Master of Science in Experimental Medicine

Department of Medicine

Edmonton, Alberta

Spring 2004

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ISBN: 0-612-96491-4

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ABSTRACT

We tested the hypothesis that GLP-2+DEX synergistically enhances lipid uptake in suckling rats when administrated directly or to their mothers during lactation or during pregnancy. We also determined if GLP-2 and DEX have late effects on lipid absorption. GLP-2 didn't change lipid uptake by direct administration, but decreased lipid uptake in the offspring of lactating or pregnant dams exposed to GLP-2. Lipid uptake increased in suckling rats when DEX was directly administered to them or was given to lactating dams. DEX given to pregnant dams decreased lipid uptake in offspring. Lipid uptake increased after one month by direct GLP-2+DEX treatment of offspring; it increased initially, then decreased in offspring of lactating dams exposed to GLP-2+DEX; and decreased in offspring of pregnant dams exposed to GLP-2+DEX. GLP-2, DEX, and GLP-2+DEX proved to have early and late effects on lipid uptake, but an additive or synergistic effect of GLP-2+DEX was not observed.

Dedication

This thesis is dedicated to my parents for their support and understanding.

Acknowledgements

I would like to particularly express gratitude to Dr. Thomson. He has provided a supportive environment for my studies and for completing this thesis. His great mentorship and relentless interest in medical research stimulated my aspiration to continue my activity in this field.

I am obliged to my thesis committee members, Dr. Bell, Dr. Cheeseman, Dr. Clandinin, Dr. Madsen, and for their comments, and suggestions related to this study. My thanks to Dr. Jones and Dr Moqbel for their suggestions during my presentation to the Graduate Education Committee.

I am thankful to Laurie Drozdowski and Elizabeth Wierzbicki for their technical assistance, friendship, and support. I am also appreciative to all the other students, Aducio Thiesen, Trudy Woudstra, Chin Chin Neo and Zoe Todd, for their contribution in my training and also in my experiments.

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LIST OF ABBREVIATIONS

11βHSD	11β-dehydrogenase enzyme
AA	arachidonic acid
ACBP	Acyl-CoA binding protein
ACS	acyl- CoA synthetase
ANXA1	annexin1
BBM	brush border membrane
BHK	baby hamster kidney fibroblast
BLM	basolateral membrane
BrDU	bromodeoxyuridine
CBC	corticosteroid binding globulin
CG	chorionic gonadotrophin
CSF	colony-stimulating factors
cAMP	cyclic adenosine monophosphate
CYP3A	cytochrome P450 3A
DEX	dexamethasone
DNA	dezoxyribonucleic acid
DPPIV	aminopeptidase dipeptidyl peptidase IV
E. coli	Escherichia coli
EGF	epidermal growth factor
EGF-R	EGF receptor
ER	endoplasmic reticulum

Erks	extracellular regulated kinase
FABP	fatty acids binding protein
FABPpm	plasma membrane-fatty acid binding protein
FAT/CD36	fatty acid transporter
FATP4	fatty acid transport protein-4
FKBP52	FK506-binding immunophilin
5-FU	5 flurouracil
GC	glucocorticosteroid
G-CSF	granulocyte colony stimulating factor
GH	growth hormone
GLP-1	glucagon glucagon-like peptide-1
GLP-2	glucagon-like peptide-2
GLP-2R	GLP-2 receptor
GLUT2	sodium-independent glucose and fructose transporter in BLM
GLUT5	sodium-independent fructose transporter in BLM
GMP	guanosine mono phosphate
GPCR	G protein coupled receptor
GR	glucocorticoid receptor
Grb2-SH2	domain SH2 of growth factor receptor bound protein
GRPP	glicentin related pancreatic polypeptide
hCG	human chorionic gonadotropin
H-FABP	heart-fatty acid binding protein
hGH	human placental growth hormone

HPA	hypothalamic-pituitary-adrenal axis
hPL	human placental lactogen
HPLC	high performance liquid chromatography
Hsp	heat shock proteins
IBD	inflammatory bowel disease
IEC	intestinal epithelial cell
I-FABP	intestinal fatty acid binding protein
IGF	insulin-like growth factor
IGF-R	insulin growth factor receptor
IGFBP	IGF binding protein
IL	interleukin
ILBP	ileal lipid binding protein
IP-1	intervening peptide 1
IP-2	intervening peptide 2
ISH	in situ hybridization
LA	linoleic acid
LCA	long chain acyl CoA ester
LCFA	long chain fatty acid
LCPUFA	long chain polyunsaturated fatty acid
L-FABP	liver fatty acid binding protein
LNA	linolenic acid
LPB	lipid binding protein
LPH	lactase-phlorizin hydrolase

LXR	liver X receptor
MAPK	mitogen-activated protein kinase
MCFA	medium chain fatty acid
MDR	multidrug resistance protein
MPGF	major proglucagon fragment
MPO	myeloperoxidase
MR	mineralocorticoid receptor
MTP	microsomal transport protein
Na^+	sodium
NHE	Na^+/H^+ exchanger
NEC	experimental necrotizing enterocolitis
OA	oleic acid
ODC	ornithine decarboxylase activity
PACAP	pituitary adenylate cyclase-activating polypeptide
PDGF	platelet-derived growth factor
PI-3K	phosphoinositol-3 kinase
PKA	protein kinase A
PKB/Akt	protein kinase B
PKC	protein kinase C
PL	placental lactogen hormone
PLA2	phospholipase A2
PUFA	polyunsaturated fatty acid
RIA	radioimmunoassay

RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RXR	retinoid X receptor
SCFA	short chain fatty acid
SFA	saturated fatty acid
SGLT1	sodium-dependent glucose transporter in BBM
SI	sucrase-isomaltase enzyme
SR-BI	scavenger receptor class B type I
T_4	thyroxine
TG	triacylglycerol
TGF-α	transforming growth factor α
TGF-β	transforming growth factor β
TNF	tumor necrosis factor
TPN	total parenteral nutrition
TRH	thyroid-releasing hormone
TSH	thyroid-stimulating hormone
TUNEL	terminal dUTP nick-end labeling
UWL	unstirred water layer
VLDL	very light density liprotein
VIP	vasoactive peptide

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Ontogeny of the intestinal tract includes all events involved in development and maturation of the gut. This complex process includes morphological maturation: transition from endodermal tube to villous-crypt architecture, and functional maturation of digestive and absorptive functions, as well as for the barrier proprieties of the mucosa (Henning et al. 1987, 1994, Sanderson and Walker 2000, Paulsen et al. 2003). Development and maturation of the intestine is the result of intricate interactions between genetic programming, internal and external stimuli. Important components include endocrine and paracrine signals, enteric nervous system, mucosa, mesenchyme, and luminal factors including bacterial flora and diet (Pacha 2000, Ferraris 2001, Nanthakumar et al. 2003).

Digestive functions of the gut exhibit age-dependent changes during the development. These ontogenic alterations are due to variations of the intestinal transporters and enzymes, as well as to changes in the permeability of the brush border membrane (BBM) (Henning et al. 1994, Sanderson and Walkers 2000, Buddington and Malo 2003). The ontogeny of lipid absorption has not yet been extensively studied.

All newborn mammals have a good correlation between the nutrients in the diet and their intestinal digestive activities. During the suckling period, the pre-duodenal digestion of lipids by lingual and gastric lipases is dominant. There is a low secretion of lipase and colipase in the newborns' pancreas, but this is compensated for by the lingual, gastric and breast milk lipases (Henning 1987).

Lipid uptake is a dual process, involving both passive diffusion and protein-mediated transport. Most of the lipid transport across the BBM is the result of passive diffusion, and only a small component is mediated by lipid protein transporters (Besnard et al. 2000, 2002, Schulthess et al. 2000). Lipid binding proteins are located on the BBM, and also in the cytosol of the enterocytes. BBM proteins identified to have a role in lipid binding include caveolin-1, scavenger receptor class B type I (SR-BI), plasma membrane-fatty acid binding protein ($FABP_{pm}$), fatty acid transporter (FAT/CD36), fatty acid transporter-4 (FATP4), and cholesterol transport protein (Schulthess et al. 2000, Thomson et al. 2003). These proteins may be involved in the transport of lipids into or across the enterocytes. Three cytosolic lipid binding proteins have been identified in the intestine: the intestinal fatty acid binding protein (I-FABP), the liver fatty acid binding protein (L-FABP) which is also found in liver, and the ileal lipid binding protein (ILBP). These proteins may take lipids away from the BBM, changing the lipid concentration at the BBM and consequently modifying the passive uptake (Thomson et al. 2003). Animal studies have shown that the uptake of lipids is higher during suckling than in adult rats, probably due to the increased fluidity of the BBM (Schwarz et al. 1985, Hubner et al. 1988), and to the more efficient absorption and metabolism of fat in suckling than in adult rats (Frost et al. 1983, Flores et al. 1989).

Hormones and peptides which contribute to the process of ontogenic development of the gut also affect the absorption rates for nutrients. Glucagon-like peptide 2 (GLP-2) and glucocorticosteroids (GC) are hormones which play a role in the ontogeny of the small intestine. Balance studies in humans have shown that GLP-2 administration improves the intestinal absorption of lipids and proteins (Jeppesen et al. 2001) and the proliferation of the small bowel mucosa in adult animals (Drucker 1996, 2003). GC treatment of pregnant animals or neonates induces precocious maturation events in the intestinal epithelia, and improves the mucosal barrier function of the offspring (Buchmiller et al. 1994, Henning et al. 1994, Sangild et al. 1995, Nanthakumar et al. 2003). However, GC have numerous adverse effects including some on the intestine, such as decreasing the crypt-villous turnover of enterocytes, and inducing atrophy of the mucosa (Batt and Scott 1982, Kritsch et al. 2000).

GLP-2 enhances sugars uptake in adult animals (Cheeseman 1996, 1997, 1998), and in a single report GLP-2 was found to enhance intestinal uptake of triolein and leucine amino acid (Brubaker et al. 1997). In adult animals, GC increase sugar (Batt and Peters 1976, Batt and Scott 1982, Scott et al. 1981, Crake et al. 1984) and lipid uptake (Thiesen et al. 2002, 2003).

This study was undertaken to evaluate 1) the influence of GLP-2, dexamethasone (DEX) and GLP-2 + DEX when administered to pregnant and lactating dams on the intestinal uptake of lipids in suckling and weanling offspring; 2) the influence of GLP-2, DEX and GLP-2 + DEX when administered to lactating dams on the intestinal uptake of lipids in suckling and weanling offspring; 3) the influence of GLP-2, DEX and GLP-2 + DEX when administered directly to suckling animals on the intestinal uptake of lipids in suckling and weanling animals; 4) whether GLP-2 and DEX have an additive or synergistic effect on lipid absorption in immature intestine; and 5) if there is a late effect of GLP-2 and DEX in post-weaning rats.

The next section presents a literature review covering lipid absorption, the ontogeny of the intestine, intestinal adaptation, the role played by GC and different growth promoting peptides, especially GLP-2, on the small intestine, and possible interactions between GLP-2 and GC.

1.2 LIPID ABSORPTION

Dietary lipids are a major source of energy and also provide structural support for many tissues. In adults in West, lipids supply between 30-40% of total dietary energy. The dietary fat contains 95% triglycerides; the other 5% includes phospholipids, sterols, and small amounts of gangliosides, fatty alcohols, suphatides, vitamins, and carotenoids (Mann and Murray 2002). Dietary lipids must be hydrolyzed and solubilized before they are absorbed in the intestine. The hydrolysis is performed by lipases from saliva, stomach, pancreas, intestine and liver. The solubilization of the lipids is carried out by the bile acids to form micelle (Duan 2000).

Lipid absorption is the sum of the mechanisms that transfer the dietary lipids from the lumen of the small intestine into the lymph and portal circulation (Schulthess et al. 2000). This complex process includes the diffusion of lipids through the unstirred water layer (UWL), transport across the BBM, binding to cytosolic proteins and diffusion across cytosol, metabolism of lipids and lipoprotein biosynthesis, and secretion of the lipids across the basolateral membrane (BLM) into the blood or lymph (Thomson et al. 2003).

Most of the lipid transport across the BBM is the result of passive diffusion, and to a smaller extent, a component mediated by lipid binding proteins. The passive diffusion of lipids across the BBM has three steps: the adsorption in the outer leaflet of the plasma membrane, the transmembrane movement, and the desorption from the cytosolic leaflet (Hamilton 1998). The rate-limiting step in the diffusion of fatty acids across the enterocytes is the trans-membrane movement of fatty acids (Kleinfeld et al. 1997, Hamilton and Kamp 1999). The permeability of the BBM is influenced by its fluidity and lipid composition. The fluidity of the BBM depends on the content of different lipids in the membrane (Meddings 1988). There is an increased BBM fluidity during the suckling period as compared with adults, because the membranes contain a higher amount of cholesterol and phospholipids per milligram protein than do the mature BBM (Schwarz et al. 1985, Hubner et al. 1988).

The maturation of the intestine is influenced postnatal by hormonal factors such as GC and thyroid hormones. GC have an important role in membrane maturation in rat intestine. The administration of GC induces a precocious maturation due to changes in the membrane fluidity, and also post-translational alterations on proteins in the BBM (Neu et al. 1986, Henning et al. 1994, Dai et al. 2002).

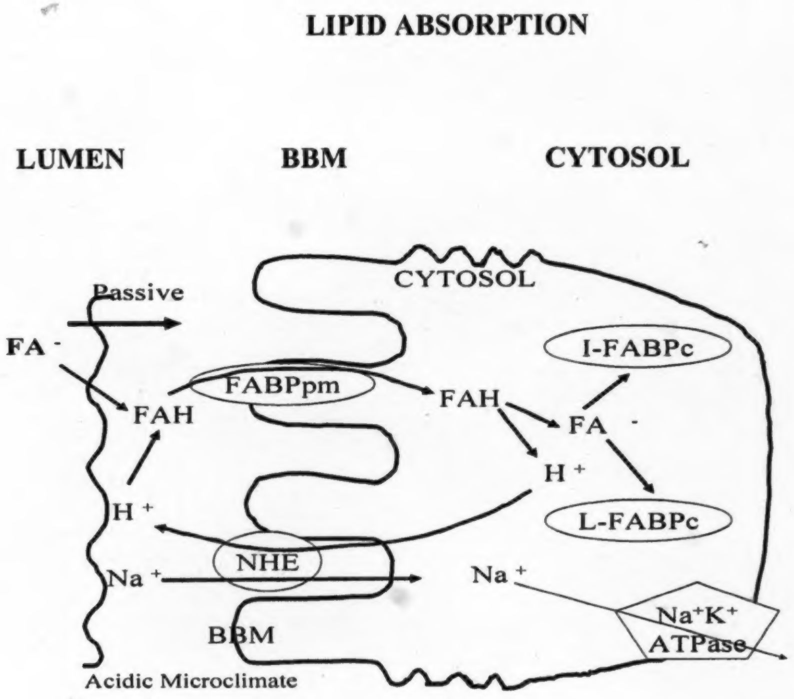
Intestinal uptake occurs predominantly in the upper third of the villus (Fingerote et al. 1994). The lipids must first cross the UWL, and than permeate the BBM. In order to correctly assess the permeability characteristics of the BBM, the experimental values must be corrected for the effective resistance of the UWL. Failure to correct for UWL resistance leads to underestimation of the permeability properties of the BBM (Thomson and Wild 2001).

The passive diffusion of lipids can be explained by three possible models (Thomson and Dietschy, 1981):

1) – The whole micelle is absorbed by BBM. There is no experimental evidence to support this model (Willson and Dietschy 1972);
2) – The collision between micelle and BBM enable the lipids to be taken up directly from the micelle into BBM. There is experimental evidence for supporting this model as suggested by the linear relationship between cholesterol uptake and bile acid concentration (Proulx et al. 1984 and Burdick et al. 1994);
3) – The lipids dissociate from the micelles into the aqueous phase of the UWL before being absorbed by BBM. The experiment which supports this latter possibility showed that fatty acid uptake decreases with an increase in the number of bile acid micelles when the concentration of fatty acids is held constant (Westergaard and Dietschy 1976). Thus, both models 2 and 3 may apply depending upon the nature of the lipids in the intestinal lumen.

The UWL provides an acidic microclimate that is maintained by the Na^+/H^+ exchanger (Schoeller et al. 1995). This acidic microclimate enables the dissociation of fatty acids from the bile acid micelle (Shiau et al. 1990) and the protonation of these fatty acids, thereby increasing their permeation across the BBM (Small et al. 1984, Clandinin and Thomson 2000). Other factors that could be involved in the rate of uptake of lipids are: the luminal lipid composition, the transposition of membrane lipids between the outer and the inner leaflet of the BBM (Devaux 1991), the membrane potential (Hollander and Morgan 1980), and the lipid binding proteins in the enterocyte cytosol or BBM (Figure 1). The cytosolic lipid transfer proteins released from lysed cells may interact with dietary lipids, thus influencing lipid uptake. It is unknown whether or not the normal enterocytes are permeable to cytosolic protein (Schulthess et al. 2000).

Figure 1. Model of lipid uptake


LIPID ABSORPTION
LUMEN
BBM
CYTOSOL
CYTOSOL
Passive
FA -
FAH
FABPpm
FAH
I-FABPc
FA -
H +
L-FABPc
H +
NHE
Na +
Na +
Na+K+ ATPase
BBM
Acidic Microclimate

Schoeller et al. 1995

FA: Fatty acids; FAH: Protonated fatty acids; NHE: Na$^+$/H$^+$ exchanger; FABPpm: Brush border membrane fatty acid-binding protein; I-FABP$_c$ intestinal fatty acid binding protein; L-FABP$_c$ liver fatty acid binding protein

BBM proteins which have been identified to have a role in lipid binding include caveolin-1, SR-BI, $FABP_{pm}$, FAT/CD36, FATP4, and cholesterol transport protein (Table 1).

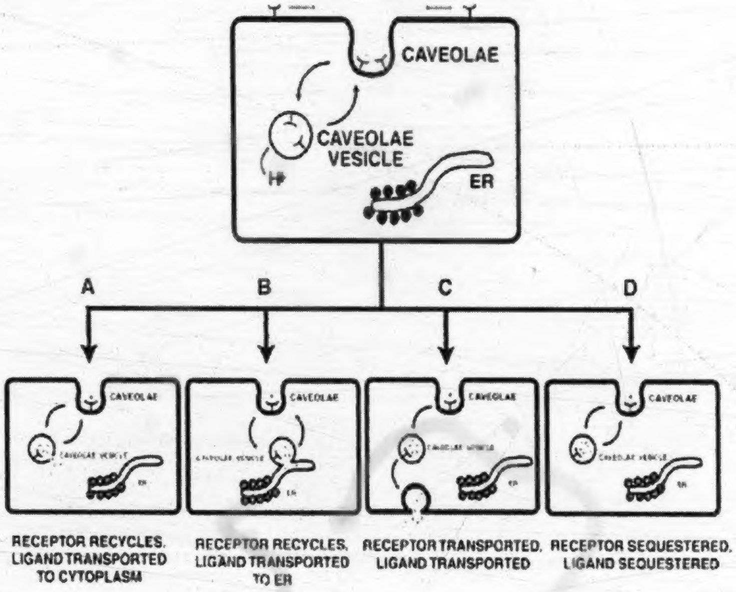
Table 1. BBM lipid binding proteins

BBM Protein	Molecular Weight (kDa)	Localization	Substrate
Caveolin-1	22	Small intestine	cholesterol and long chain fatty acids (LCFA)
SR-BI	57	Liver, peripheral tissue, small intestine	high-density lipoproteins, phospholipids, triacylglycerol, cholesterol and cholesterol esters
FABPpm	40	Adipose tissue, heart, liver, intestine	LCFA
FAT/CD36	88	Adipose tissue, heart, skeletal muscle, spleen, intestine	LCFA, triglycerides
FATP4	63	Small intestine	LCFA (oleate)
cholesterol transport protein	145	Small intestine	cholesterol

Caveolin-1 is a 22 kDa integral protein found in an invagination of the plasma membrane, which constitutes the caveolae. Caveolae have a unique structure, being composed from cholesterol and sphingolipids, as compared with plasma membranes that are composed largely by phospholipids. This composition enables caveolae and caveolae related domains to form a liquid-order phase in membranes (Smart et al. 1999).

The caveolae membrane system is involved in endocytosis (which is the transcellular transport between the two surfaces of the cell) and in potocytosis (potocytosis is a mechanism for small molecule uptake independent of an endocytic process). A molecule binds to a receptor in a flat or open caveolae. The caveolae then invaginates, and may transiently form a sealed compartment independent of the extracellular space, but still remains contiguous with the plasma membrane. The formation of a sealed microenvironment facilitates the uptake of the molecules across the plasma membrane. The invaginated caveolae then flattens or opens, and the cycle is repeated (Anderson et al. 1998, Smart et al. 1999, Liu et al. 2002).

Figure 2. Pathways of potocytosis


CAVEOLAE
CAVEOLAE VESICLE
H+
ER
A
B
C
D
CAVEOLAE
RECEPTOR RECYCLES, LIGAND TRANSPORTED TO CYTOPLASM
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Anderson RGW 1998

Caveolin-1 is needed for the internalization and trafficking of the caveolae (Anderson 1998). Caveolin-1 has binding affinity for long-chain fatty acids (LCFA) (Trigatti et al. 1999), and for cholesterol (Field et al. 1998). Caveolin-1 may act as storage place for cholesterol, and may play a role in the sterol-sensing component of the BBM (Field et al. 1998). Also, caveolin-1 may participate in the intracellular targeting of cholesterol (Uitenboogaard et al. 2000). Furthermore, caveolin-1 may play a role in signal transduction, as suggested by the interaction of calveolin-1 with G-protein, Ras and Src kinases, or epidermal factor receptor (Okamoto et al. 1998).

SR-BI is a 57 kDa protein identified in both BBM and BLM, especially in the jejunum, and in negligible amounts in ileum (Cai et al. 2001). SR-BI serves as a docking receptor mediating the flux of lipid molecules. Interestingly, the flux of lipids is bidirectional; the lipid molecules move from the donor particle to the BBM, and back again (Schulthess et al. 2000).

The localization of SR-BI on the BBM of the jejunum is consistent with the hypothesis of its possible role in dietary cholesterol absorption, whereas SR-BI present on the BLM of the ileum suggests its possible involvement in intestinal lipoprotein uptake (Cai et al. 2001).

FABP_{pm} is a 43 kDa protein located in the BBM and BLM (Stremmel et al. 1985). FABP_{pm} binds monoglycerides, LCFA and cholesterol. The role of FABP_{pm} in absorption of LCFA was demonstrated by the inhibition of [^{3}H]-oleate uptake in jejunal explants or sheets of intestine using monospecific FABP_{pm} antibody (Stremmel 1988, Schoeller et al. 1995). The uptake of oleic acid by FABP also involves the activation of Na^+/H^+ exchanger (Schoeller et al. 1995).

Fatty acid translocase, FAT, is a 88 kDa glycoprotein found in tissues with high fatty acid metabolism such as adipocytes, cardiomyocytes, myocytes, mammary gland cells and enterocytes (Besnard and Niot 2000). FAT was firstly identified in rat adipocytes (Amburad et al. 1984), and it has an 85% homology with human scavenger receptor CD36 which is found in platelets, monocytes, and endothelial cells. In the intestine, FAT/CD36 is located in BBM in the upper two third of the villi, at the major sites of lipid absorption in the duodenojejunum (Poirier et al. 1996). FAT binds to negatively charged fatty acids; therefore, it might constitute a high affinity, low capacity transport system for ionized LCFA (Baillie et al. 1996). The effect of FAT/CD36 on transport seems to require as partner the cytosolic FABP (Van Nieuwenhoven et al. 1998). FAT/CD36 might be a lipid sensor in the intestine. Increases in both FAT mRNA levels and cytosolic L-FABP are reported after mice are fed high lipids diet (Poirier et al 1997). A diet rich in polyunsaturated fatty acids (PUFA) also increases the expression of FAT mRNA (Poirier et al. 1996).

FATP is a 63 kDa protein found in different isoforms, five in rats and, respectively six in humans (Hirsch et al. 1998). FATP4 is the isoform expressed in the intestine of humans and FATP1 is found in murines. FATP4 is located in the BBM of the villi in the jejunum and ileum, with lower levels in the duodenum. FATP4 is involved in the transport of medium- and long-chain fatty acids (Stahl et al. 1999). FATP from adipocytes is co-expressed in plasma membrane with long chain acyl-CoA synthetase (ACS); this system increases the efficiency of LCFA uptake (Gargiulo et al. 1999). It is not known whether the intestinal FATP has a similar system.

The 'cholesterol transport protein' is a 145 kDa integral protein present in BBM of rabbit enterocytes (Kramer et al. 2000). Another transporter that may also be involved in the intestinal uptake of cholesterol is the multidrug resistance protein (MDR) (Tessner and Stenson 2000). The existence of a transporter protein for cholesterol was suggested as early as 1990 by Thurnhofer and Hauser. This conclusion resulted from the experiments in which the rate of cholesterol uptake was reduced by 80% after proteolytic treatments of the BBM (Thurnhofer and Hauser 1990). Interestingly, the hydrolysis of cholesterol esters is not a prerequisite for cholesterol absorption by cholesterol transporters. The uptake of cholesteryl oleate by the BBM is as effective as that of free cholesterol. Moreover, the kinetics of cholesteryl oleate uptake was similar with the kinetics of an analog which is resistant to cholesterol esterases. Based on these experiments, it was concluded that the absorption of cholesterol and cholesterol esters in the BBM work side by side depending on conditions in the lumen of the small intestine (Compassi et al. 1995).

There is also efflux of cholesterol from cells (Jogchum and Mensink 2002). Cholesterol absorption is reduced by plant sterols (Subbiah 1973; Vahouny et al. 1981; Ikeda et al. 1988; Becker et al. 1993). This action of plant sterols was initially explained by physicochemical effects of these compounds, which are in competition with cholesterol in their incorporation into micelles, and in the uptake from bile salt micelles (Ikeda et al. 1988). More recently, the hypocholestolemic effect of plant stanols has been explained by the presence of ATP binding cassette (ABCA1) transporters of cholesterol in BBM of intestinal mucosa cells (Jogchum and Mensink 2002). ABCA1, as well as the other ABC transporters, belong to the ABCG transporter family which has been shown to be involved in the regulation of lipid traffic in macrophages, hepatocytes and enterocytes (Schmitz et al. 2001). There is some evidence that ABCA1 mediates the efflux of cholesterol back into the intestinal lumen. Interestingly, the ABCA1 expression is regulated by a nuclear receptor system formed by mandatory heterodimers between the liver X receptors (LXR) and retinoid X receptors (RXR). LXR binds cholesterol or plant sterols and RXR binds rexinoids (Repa et al 2000). Experiments with LXR/RXR heterodimers revealed that they regulate the expression of the ABCA1 transporter, and thus LXR/RXRs regulate the level of cholesterol in enterocytes (Jogchum and Mensink 2002).

In enterocytes, the cytosolic lipid binding proteins bind and transport lipids and bile acids. Three of them have been identified in the intestine: I-FABP, L-FABP which is also found in liver, and ILBP (Table 2).

Table 2. Intracellular lipid binding proteins

Intracellular Proteins	**Molecular Weight (kDa)**	**Localization**	**Substrate**
L-FABP	14-15	liver and small intestine	LCFA, heme, bile acids, acyl CoA
I-FABP	14-15	small intestine	LCFA
ILBP	14	ileum (predominant in distal ileum)	bile acids
ABCB	10	liver, heart muscles, adipose tissue, intestine	medium- and long-chain acyl-CoA esters
MTP	59	all tisues	TG, phospholipids, cholesterol

I-FABP and L-FABP are expressed along the villi in both the jejunum and ileum (Poirier et al. 1996). I-FABP is a 15.1 kDa protein, found most abundantly in the distal ileum and at the villous tip (Besnard et al. 2000). L-FABP is a 14.1 kDa protein present mainly in the proximal jejunum at the crypt–villous junction. L-FABP is absent at the villous tips (Iseki and Kondo 1990). I-FABP has a greater affinity for saturated than for PUFA (Cistola et al. 1989). In contrast, L-FABP exhibits a greater affinity for PUFA than for saturated fatty acids (SFA) (Cistola et al. 1989). I-FABP binds protonated fatty acids, while L-FABP binds unprotonated fatty acids (Cistola et al. 1989; Thomson et al. 2002). I-FABP transports fatty acids and releases them by a collisional interaction with membranes, which suggests a role in the uptake or targeting fatty acids. L-FABP transfers fatty acids in an aqueous diffusion mediated process, which suggests their having a role as a buffer for fatty acids in cytosol (Hsu and Storch 1996, Besnard et al. 2002, Agellon et al. 2002).

To study the role played by I-FABP in lipid absorption, several experiments have been performed with I-FABP knockout mice. Body weight gain was used as an indicator of dietary fat assimilation. The knockout male mice that were fed a low-fat diet or high-fat diet were consistently heavier than their normal counterparts. In contrast, the knockout female mice fed a low-fat diet were indistinguishable from normal mice. Only the knockout female mice fed a high-fat diet gained less weight than did the normal female mice. Measurements for lipid content in plasma and organs were also performed. Cholesterol, triacylglycerol (TG), glucose and insulin were measured in the plasma. Samples of liver and epididymal fat pads were used to determine cholesterol and TG content in tissuesand theire values were normalized to grams of organ weight.

Although I-FABP has been suggested to be involved in the absorption of dietary fatty acids, the results of this study suggest that I-FABP is not imperative for fatty acid uptake. In fact, the higher concentrations of TG in the plasma of male knockout mice, which is consistent with their greater weight gain, implies that the rate of dietary fat transfer into the plasma compartment in male mice is actually increased in the absence of I-FABP (Vassileva et al. 2000, Agellon et al. 2002).

The mRNA abundance for both I-FABP and L-FABP was enhanced by feeding rats with a PUFA enriched diet (Poirier et al. 1996). The mRNA expression was also enhanced in starved rats (Besnard et al. 1991). It is possible that L-FABP influences growth and differentiation of enterocytes (Besnard et al. 2002, Thomson et al. 2003). L-FABP binds not only to fatty acids but also to growth factors, prostaglandins and leukotrienes; L-FABP participates in regulation of genes as a partner of the peroxisome proliferator-activated receptors (PPAR).

The mechanism and regulation of the uptake of extracellular LCFA into mammalian cells is not well understood. PPAR play unique roles in lipid homeostasis. PPAR are part of the nuclear hormone receptor superfamily, and three subtypes have been described, namely α, β, γ. They have unique tissue distribution patterns. Furthermore, the roles of PPAR in mediating changes in gene expression appear to be cell- and tissue-specific. Also, the type of agonist influences the magnitude of their responses. In the intestine for example, activation of PPARα by feeding rats with a diet containing Wy 14,643 (a hypolipidemic drug) resulted in a large induction of I-FABP mRNA, FAT mRNA, FATP mRNA, and peroxisomal HD mRNA (the second enzyme of the peroxisomal beta-oxidation pathway) (Motojima et al. 1998). PPARα may be involved in up-regulating the expression of L-FABP and I-FABP genes in murine liver (Motojima et al. 2000), in addition to the known induction of hepatic peroxisomal lipid-metabolizing enzymes (Motojima et al. 1998). The experiments with rats treated with bezafibrate, a PPAR hypolipidemic drug, showed an increased amount of L-FABP protein and mRNA in the liver of these rats (Besnard et al. 1993) and an increase of mRNA of FAT. These findings suggest a complementary role of FAT and FABP (Poirier et al. 1996; Van Nieuwenhoven et al. 1998). PPARα activation increases the cytosolic transport and the oxidation of lipids. PPARα mediates fibrate and dietary PUFA induction of hepatic peroxisomal lipid-metabolizing enzymes, including acyl-CoA oxidase, a key enzyme in the regulation of peroxisomal lipid catabolism (Motojima et al. 1998). Interestingly, the PUFA activates PPAR as potently as does the hypolipydemic drug Wy 14,643 (Keller et al. 1993). In addition, the binding of PUFA to PPAR is increased compared with other fatty acids (Murakami et al. 1999).

ILBP is a 14 kDa protein expressed mainly in the cytosol of enterocytes in the distal ileum. In rats, the ILBP amino acids sequence has a 25 % homology with L-FABP and I-FABP, but they are immunological distinct. The ileum is the only site of active bile acid absorption that is coincident with the localization of ILBP mRNA expression (Gong et al. 1994). ILBP exhibits an increase in the affinity/capacity when it is stimulated by bile acids. Therefore, the ileal bile acid reabsorption system can be modulated by an intracellular increase of ILBP affinity/capacity which allows the maximal adaptation of transport activity to changing substrate loads (Kramer et al. 1998).

The cytosolic acyl-CoA binding protein (ACBP) plays a role in the binding of long chain fatty acids (Kragelund et al. 1999; Besnard and Niot 2000). ACBP is a 10 kDa protein which binds long chain acyl CoA esters (LCA). ACBP binds LCA with high affinity, and may play a role in LCFA transport and metabolism (Kragelund et al. 1999). ABCP regulates the levels of LCA in the cell, and has a role in cellular signaling (Kragelund et al. 1999; Besnard et al. 2000). ACBP and LCA are involved in signal transduction and gene regulation (Kragelund et al. 1999; Besnard and Niot 2000).

The microsomal transport protein (MTP) is a heterodimer involved in the transfer of triglyceride, phospholipids and cholesterol esters in apolipoprotein B (apo B) (Lin et al. 1994). This heterodimer contains a 59 kDa multifunctional subunit, identical to protein disulfide isomerase which is linked to the endoplasmic reticulum (ER), and a 97 kDa subunit with lipid transfer activity (White et al. 1998). MTP also plays a key role in very light density lipoproteins (VLDL) synthesis. The absence of MTP in mutant MTP humans causes abetalipoproteinemia and the absence of VLDL in the plasma (Wetterau et al. 1992). The major clinical consequence of this disorder is the deficiency of essential fatty acids and fat-soluble vitamins (White et al. 1998).

The rate-limiting step in lipid absorption may be the transport of TG from the ER to the Golgi. The intestine has two systems which enable it to absorb dietary lipids. The first one is the binding of lipids to cytosolic binding proteins, and the second one is the chylomicron secretion after re-synthesis of TG from absorbed fatty acids and monoacylglycerol. The capacity of the intestine to export chylomicrons is limited. The formation of the ER vesicle that transports the developing chylomicrons to the Golgi parallels the capacity of chylomicron secretion of the intestine . The rates of prechylomicron vesicle formation determine the rate of lipid absorption (Mansbach and Dowell 2000, Clandinin and Thomson 2000).

Lipid uptake is a dual process involving passive diffusion and protein-mediated transport. It is not known yet which system contributes more to lipid absorption (Besnard et al. 2000, Shulthess 2000). The small intestine is subjected to large variations in lipid supply from the diet, bile, and from sloughed intestinal cells. It is possible that passive diffusion may be a high-capacity, low-affinity system that operates during high lipid loads, and that the lipid binding proteins may constitute a low-capacity, high-affinity system that works at low lipid concentrations (Besnard and Niot 2000). Thus, the presence in the enterocyte of more than eight lipid binding proteins raises the question of the physiological role of these proteins. Perhaps, they may be involved in specific uptake and/or selection of fatty acids, and may play a role in fatty acid targeting as well as in cellular signaling (Besnard and Niot 2000).

1.3 ONTOGENY

The ontogeny of the intestinal tract includes all the events involved in the development and maturation process of the gut. This complex process includes morphological maturation, the transition from the endodermal tube to the villous-crypt architecture, a functional maturation for digestive and absorptive functions, as well as for the barrier proprieties of the mucosa (Henning et al. 1994, Sanderson and Walker 2000, Paulsen et al. 2003). The ontogeny can be divided into four stages: a) morphogenesis; b) cellular differentiation and fetal period; c) the suckling period; and d) the weaning period.

The main morphologic development of the gastrointestinal tract occurs during the fetal period after the embryo folds the trilaminar germ disc. Initially, two invaginations of the endoderm emerge: the first at the anterior end of the embryo and the second at the posterior end. Thus, the gut tube consists of cranial and caudal blind-ending tubes that will connect later during the developmental process. The epithelium of the intestine is derived from endoderm and ectoderm, and the gut smooth muscle is derived from mesenchyme. The primitive tube forms the foregut, midgut and hindgut. The foregut gives rise to the esophagus, lung, thyroid, stomach, duodenum, liver, pancreas and gallbladder. The midgut rotates during embryogenesis, and develops into the small intestine and colon. The hindgut gives rise to the rectum and the urogenital sinus (Larsen 2001).

Animal models have been used to study the details about the embryologic developments of the gastrointestinal tract. Because in these models the maturation of the gastrointestinal tract occurs during the postnatal period and not during the fetal period, as it does in humans, animal models offer an advantage in studying the maturation processes. Also, the animal models facilitate an easier monitoring of the maturation processes, because of a shorter duration of gestation than in humans. For example, in humans the duration of gestation is nine months, in rats 21 days and in pigs 105 days. In humans, the primitive tube is recanalized between the seventh and the eighth week of gestation, the microvilli emerge, the mucosa differentiates from a single layer of cuboidal- to the columnar-epithelium by the end of the ninth week, and the villi and the crypts develop between the eighth and the twelfth week of gestation. In rats the primitive tube is recanalized in the 14th day, the stratified epithelium occurs in the 15th day, the villi formation takes place during the 18-19th day of gestation (Sanderson and Walker 2000), and the crypts develop after birth (Pacha 2000). In pigs, the primitive tube is formed at 30 days, the villi emerge in 35 days, and the crypts are formed in 60 days (Kelly and King 2001).

The maturation of the intestinal functions follows a similar pattern, with some differences between species. In humans, the sucrase-isomaltase enzyme (SI) and the SI mRNA appear in the ninth to tenth week, but their levels are low until birth. The lactase-phlorizin hydrolase (LPH) and the LPH mRNA appear with the emerging villi in the 12th week, increase until birth, than decrease after weaning. In rats, the SI mRNA appears at day 16-17 postnatally, and SI increases in activity at the weaning time; LPH expression and mRNA occur during 14-15th gestational days, and decrease at weaning (Sanderson and Walker 2000). In pigs, the maturation process is intermediary between rats and humans: the LDH activity and mRNA are detected in 7 week old fetuses, and increase at birth until weaning at three to five weeks (Kelly and King 2001). It is not known when the SI enzyme appears for the first time in pigs, but the low level in the fetus continues to rise from birth to weaning (Buddington and Malo 1996, Kelly and King 2001).

The ontogeny of the intestine is a result of intricate interactions between internal influences of the genetic programming, and external stimuli such as endocrine and paracrine signals, the enteric nervous system, the mucosa, the mesenchyme, and luminal factors including bacterial flora and diet. The intercellular communications among epithelial cells, extracellular matrix, and mesenchyme are central to cytodifferentiation and proliferation (Henning et al. 1994, Pacha 2000, Kedinger et al. 1998, 2000). The genetic programming controls in a time- and space-specific manner the gene expression. There are large changes in gene expression along the cephalo-caudal axis and the crypt-villous axis. The changes in gene expression are regulated by the homeobox genes (HOX), and by the dispersed homeobox genes CDX1, CDX2 and PDX1 (Beck et al. 2000, Clatworthy and Subramanian 2001). CDX1 and CDX2 expressions are restricted to the gastrointestinal tract (Beck et al. 2000). CDX1 and CDX2 are involved in the positional information and/or in the control of differentiation and proliferation. CDX1 is expressed in the crypts, and CDX2 is expressed in the differentiation compartment. Recently, it has been found that the CDX genes influence and interact with the regulatory elements of the HOX genes (Sanderson and Walker 2000). The mechanism of activation and transcription of these genes is more complicated. For example, hepatocyte nuclear factor-1α, GATA-4 protein, CDX1 and CDX2 proteins modulate the SI gene promoter activity. The genetic programming controls the development, while the external factors are potential regulators of proliferation, differentiation and migration. If fetal intestinal grafts are transplanted under the kidney capsule of adult rats, the ontogeny of these isografts is unchanged (Duluc et al. 1994, Pacha 2000, Nanthakumar et al. 2003).

Studies of digestive functions during suckling and weaning show an age-dependent change in the abundance and/or activity of transporters and enzymes. The hormones or the diet have a small role in modulating the transporters or the enzymes during this period (Pacha 2000, Ferraris 2001). The best known hormones and peptides contributing to the ontogenic development of the gut are: GC, thyroid hormones, insulin, epidermal growth factor (EGF), insulin-like growth factor (IGF), glucagon, glucagon-like peptides GLP-1 and GLP-2, polyamines, gastrin and vasoactive peptide (VIP).

The serum levels of some hormones change during development, and may influence the ontogeny (Fuller et al. 1990). The blood levels of binding proteins for some hormones vary during development. For instance, the GC bind to corticoid binding globulin (CBG), and this binding increases or decreases the activity of the GC at different developmental stages (Myles et al. 1974, Leeper et al. 1988 and Solomon et al. 2001). GC, like other nuclear hormones, act mainly through genomic effects after deoxyribonucleic acid (DNA) binding inside the nucleus of the cells. GC in physiological doses have a trophic effect on the intestine growth (Foligne et al. 2001), and influence all tissues directly or indirectly (Baxter et al. 1979). There is also an ontogeny of the receptors for all these hormones, which are expressed and/or become active at different times of development, including the GLP-2 receptor (GLP-2R) that has its own alteration from the fetal to the adult stage, and this change is variable in different species (Table 3).

Digestive functions also have their process of ontogenic development. Fetuses extract nutrients from the placenta, and some intestinal transporters are active before birth. The prenatal transporters are important in normal fetal development (Sanderson and Walker 2000). At birth, all mammals have a good correlation between the nutrients in the diet and their intestinal digestive activity.

Table 3. Ontogeny of the receptors on mucosal cells of the intestine

Ligand	Species	Characteristics of the receptor
Glucocorticosteroids	rat	Binding capacity increases from fetal life to the end of the first 2wk, then decreases until adult hood
Thyroid hormones	rat	constant binding capacity
EGF	rat	Binding capacity decreases after birth and reaches adult levels after first 2 postnatal days
IGF-1	rat	Binding capacity increases during suckling and weaning with a subsequent decrease
IGF-2	rat	Binding capacity decreases age dependent from suckling to adulthood
GLP-2	Rat	mRNA of the receptor is higher in fetal and neonatal intestine than in adult
GLP-2	Pig	GLP-2R expressed in fetal and neonatal intestine, but GLP-2 had no effect on gut growth in fetuses

Pacha 2000, Lovshin et al. 2000, Burrin et al. 2000, Petersen et al. 2001

Rat milk, like that of humans and pigs, contains lactose as the main carbohydrate. Lactose is digested in the intestine by the BBM disaccharide enzyme lactase-phlorizin hydrolase (LPH), which is present at least until weaning (Henning et al. 1994).

The prenatal expression of the active transport pathways allows the fetus to obtain carbohydrates, amino acids and proteins from the swallowed amniotic fluid. This system allows the fetus to acquire 10% to 15% of its energy. The major source of amniotic fluid is the amnionic epithelium layer, but also the fetus swallow, inhale and urinate into the fluid. The amount of proteins in amniotic fluid is similar with the serum, but glutamine concentrationincreases when the fetus starts swallowing amniotic fluid, and this is critical for the intestinal metabolism (Wu et al. 1996).

Glucose absorption in human, rat, or pig fetuses has two transport systems: a high-affinity low-capacity system, mediated by the sodium (Na^+)-dependent glucose transporter in BBM (SGLT1), and a low-affinity high-capacity system. SGLT1 appears in humans in the first trimester, in rat ileum in the 19th day of gestation, and in pigs after the 30th day of gestation (Sanderson and Walker 2000). In pigs, the activity of the low-affinity glucose transport system declines at birth. In rodents, both systems coexist after birth, but the low affinity system disappears at weaning (Sanderson and Walker 2000). Studies on rats, rabbits, and chickens showed that the capacity of the active transport system increases 10 days after birth to reach a peak, than decreases to the capacities observed in the adult (Younoszai et al. 1975, Buddington and Diamond 1989). This suggests that the decline is not caused by a loss in the number of transporters present at birth, but instead is due to the combination of mature enterocytes with immature enterocytes (Sanderson and Walker 2000).

The sodium-independent glucose and fructose transporter in BLM (GLUT2), follows the expression of SGLT1. In contrast with the adult intestine, the fetal intestine in rats and humans has two transporters: GLUT2 and GLUT1. GLUT1 appears before GLUT2, and declines during fetal life (Pacha 2000). Fructose uptake appears before birth, is low during the suckling period, and rises significantly at weaning. The activity of the sodium-independent fructose transporter in BLM (GLUT5) is programmed at weaning to match the fructose levels from the normal diet of that species (Buddington and Diamond 1989).

The amino acid transporters emerge in the fetal period in the intestine of chick, rat, rabbit and humans (Younoszai et al. 1975). During the suckling period, dietary proteins may be intactly absorbed. Amino acid transporters are present from birth, and are more abundant during suckling than adulthood (Younoszai et al. 1975, Toloza et al. 1992). Amino acid uptake reaches a peak at birth, decreases at weaning, and continues to decrease in adult animals. The uptake of essential amino acids decreases more than that of the nonessential amino acids. This decline matches the protein requirements in young and adult animals (Buddington and Diamond 1989, Buddington and Malo 2003).

The absorptive capacities for a number of minerals such as iron, calcium, copper, zinc and magnesium decline at weaning, and their uptake is switched from a nonsaturable, passive mechanism to a transporter-mediated process (Menelley et al. 1982, Henning 1987). This high capacity for mineral uptake is important during the period of rapid growth. The absorption of metals is also favored by their high availability in milk (Henning et al. 1994).

Newborns have higher fecal fat loss than do older infants and adults. The capacity to absorb lipids is decreased in the postnatal period by the lower pancreatic and intestinal lipase activity (Pacha 2000). During the suckling period, the pre-duodenal digestion of lipids by lingual and gastric lipases is dominant. Rats and mice have lingual lipase and no gastric lipase, whereas humans have high levels of gastric lipase and low amounts of lingual lipase (Duan 2000). The exocrine pancreatic secretion has small amounts of digestive enzymes during the first two weeks of life. The amounts of trypsin, chymotrypsin, amylase and lipase increase significantly in weanling rats. The low secretion of lipase and colipase by the newborn pancreas is compensated for by the lingual, gastric and breast milk lipases (Henning 1987).

Most of the lipid transport across the BBM is the result of passive diffusion (Hamilton 1998). The permeability of the rat intestine is greatest during suckling, and decreases at the weanling time. The decline in the permeability of the BBM occurs due to changes in the membrane constituents (Schwarz et al. 1985, Hubner et al. 1988). Because of the increased BBM permeability, lipid uptake is increased during suckling. Animal studies have shown more efficient dietary fat absorption and metabolism in suckling than in adult rats (Frost et al. 1983, Flores et al. 1989). The rate of TG secretion from the small intestine into lymph and plasma is also higher than in adult rats. The suckling rat, despite its high-fat milk diet and its very efficient absorption of dietary lipids, does not transport dietary lipids into the lymph as efficiently as does the adult rat (Ee et al. 2000).

The intracellular fatty acid binding proteins (FABP) have their own ontogeny. The genes for both intestinal (I-) and liver (L-) FABP are expressed in the fetal stage by the 17th –18th day. Interestingly, the I/L-FABP mRNA are produced in the same cells from which the intestinal villi emerge (Rubin et al. 1989). In contrast, BBM lactase and other markers of fetal intestinal mucosa are homogenously distributed (Rubin et al. 1989, 1992). Between day 1 and 7 after birth, L-FABP mRNA is abundant at the villous base, and decreases in cells located in the upper and middle third of the villus, but the protein is still expressed homogenously from the villous base to tip (Henning et al. 1994). This restricted mRNA production of L-FABP mRNA may be caused in postnatal life by the replacement of fetal epithelium with a new enterocyte population. mRNA of ILBP is initially expressed on postnatal days 12-14, and rises continuously from the suckling period to adulthood. The level of ILBP mRNA is highest in the ileum and distal jejunum, and lower in other regions of the small intestine (Henning et al. 1994).

Generally, postnatal changes in nutrient absorption are relatively small as compared with the changes in digestive enzymes. The activity of LPH decreases during suckling, whereas the activity of sucrase-isomaltase (SI) suddenly rises at weaning (Duluc et al. 1992, Henning et al. 1994). LPH activity is correlated with its mRNA, and this activity is transcriptionally regulated (Fajardo et al. 1994). LPH activity decreases at weaning and is associated with a fall in mRNA levels. Posttranslational regulatory mechanisms are involved in the decline in LPH activity, and this may be modulated by thyroxine (Liu et al. 1992). In humans, LPH mRNA activity is first detected in fetal intestine at 24 weeks. The mRNA activity increases at 32 weeks of gestation, and remains at high levels until birth, then falls to lower levels in the first year of life (Sanderson and Walker 2000).

The SI activity and/or the mRNA are not present in suckling rats (Leeper and Henning 1990). In mice, SI mRNA is expressed in small amounts until weaning (Tung et al. 1997). At weaning, there is a dramatic increase in SI activity, first in the enterocytes located in the crypt-villous junction and in the proximal intestine. The dramatic increase in SI mRNA and activity appears to be genetically programmed (Henning et al. 1985). The level of SI activity follows the mRNA expression, which suggests control at the level of transcription. The SI gene promoter initiates transcription after it interacts with hepatocyte nuclear factor-1α, GATA-4 protein, CDX-1 and CDX-2 (Boudreau et al. 2002). Although the SI gene expression appears to be genetically regulated, premature SI induction can be produced in the immature rat intestine by GC and thyroid hormones (Henning et al. 1985, Nanthakumar et al. 2003). Thyroxin (T_4) alone has no effect on intestinal maturation, and GC combined with T_4 work together to increase the activity of SI.

There is not a general synergism between GC and T_4. For example, LPH levels during suckling are not affected by the combined treatment (Henning et al. 1994). The administration of GC during suckling facilitates the induction of the same enzymes as those which are increased by carbohydrate feeding (Sangild et al. 1995). Interestingly, the activity of the SI enzyme and galactose absorption in adults can also be enhanced by treatment with GC or GLP-2 (Batt and Peters 1976, 1982, Kitchen et al. 2000). In humans, GC does not affect the expression of SI *in utero*, although in cultured cells GC and the combination of GC and thyroxine proved to increase SI expression per cell (Sanderson and Walker 2000). The activity of other BBM enzymes such as maltase and alkaline phosphatase is also dramatically altered at weaning. Maltase levels are low at birth and increase at weaning (Galand et al. 1989). At weaning alkaline phosphatase increases in the duodenum and decreases in the ileum (Henning et al. 1994). The amount of alkaline phosphatase enzyme initially increases at birth after fat feeding. It was suggested that the enzyme may play a role in regulating paracellular pathways of absorption, but there were no experimental evidences to support this theory (DeSchryver-Kecskemeti et al. 1991).

1.3.1 Diet influence on ontogeny

Dietary changes may act as regulators for the ontogenetic program controlling intestinal development. Diet plays a secondary role in controlling the BBM enzyme levels at weaning (Henning et al. 1994). SI levels are not modified by differences in the sucrose content of the diet at weaning (Henning and Guerin 1981). In rats, LPH activity decreases precociously by early weaning, but the reduction of LPH mRNA is observed only in the ileum. A delay in weaning postpones decrease but does not prevent the normal decline in LPH activity, and delays the mRNA decrease only in the ileum (Duluc et al. 1992). These results suggest that the ontogenic process at weaning is primarily preprogrammed, and is only slightly influenced by diet (Henning et al. 1994).

The capacity to modulate some of the transport functions in relation to the dietary content and load appears to be already reached at weaning. Glucose transport and levels of SGLT1 mRNA are not affected during suckling by changes in dietary glucose levels. The levels of GLUT2 mRNA in weanling rats also do not change with dietary carbohydrate levels. In contrast with glucose transport, intestinal fructose uptake and GLUT5 mRNA can be precociously enhanced by dietary fructose or sucrose. In young rats, the responsive age is between 15 and 24 days old, and earlier exposure of the intestine does not alter uptake (Sanderson and Walker 2000). These data suggest that the prenatal sugar transporters such as SGLT1 or GLUT2, which are expressed before birth (the early-onset transporters), may not be modulated during suckling by dietary or endocrine signals. Transporters expressed after birth (the postnatal transporters) such as GLUT5, may be regulated by diet or by endocrine signals (Sanderson and Walker 2000, Ferraris 2001).

1.3.2 Milk and milk-borne hormones in ontogeny

The main role of milk is to supply the nutrients necessary to the development of the suckling offspring. Milk contains water as the major component, as well as lipids, proteins, lactose, minerals, vitamins, antibodies and leukocytes. There are differences between and within species in the percentage of these milk components. Lipids are mainly represented by TG and are the major source of energy for the young (Casey and Hambidge 1983, Martin et al. 1993). About 97% of fats in rat milk are TG, out of which 35% are medium chain fatty acids (MCFA). The MCFA are preferentially used in the liver of suckling rats (Fernando-Warnakulasuriya et al. 1981). There are various types of proteins in milk, and they offer primarily the amino acid pattern necessary for growth. The most abundant and distinctive of these proteins is casein. Lactose, a disaccharide, is the major sugar in milk. It is composed of galactose and glucose, and is peculiar to the secretion of the mammary gland. Milk contains large quantities of calcium, phosphorus, potassium and magnesium, as well as trace minerals. These minerals are necessary for the development of the skeleton of the offspring. The fat soluble vitamins A, D, E, and K are found in milk, as well as vitamin B (Casey and Hambidge 1983). Many diverse compounds are delivered with milk including cells such as maternal leukocytes (Goldman et al. 1997).

The percentage of all these compounds varies in milk during the suckling period. For example, during the process of lactation, the levels of TG and MCFA increase, whereas the levels of cholesterol and LCFA decrease (Boersma et al. 1991). In all species, the milk of the first day after birth, known as colostrum, is rich in proteins with specific functions: immunoglobulins, antimicrobial peptides, lactoferrin and growth factors (Playford et al. 2000). Milk composition changes in the next few days to a solution rich in lactose and with moderate protein levels (Neville et al. 1983). Evaluating the milk in humans and cows it was measured that the content of water and fat is similar, but human milk contains less protein 0.9% and more carbohydrates 7% than does cow's milk 2.9%, respectively 4.8%. More profound differences include the higher nonprotein nitrogen content in human milk. Interestingly, the human colostrum contains almost similar compound as cow milk (Jensen et al. 1990). Milk secretion involves two main processes in the mammary gland: lactose and fatty acid synthesis. The fatty acids from milk are derived from two sources: the biosynthesis of fatty acids within the mammary gland, and the uptake of fatty acids from the plasma (Del Prado et al. 1999). The fatty acids synthesized by the mammary gland are MCFA (Spear et al. 1992). Diet of the mother can alter the constituents of the milk by changing the pattern of milk fatty acids. For example, feeding nursing rats with corn oil (increased PUFA concentration) caused an increase in the mother's milk in PUFA and MCFA; feeding rat dams with hydrogenated corn oil (increased SFA concentration) caused a decrease in LCFA content of the milk (Ross et al. 1985).

There are some contradictions in experimental results that could be explained by differences between species. In nursing rats fed with a high-fat concentrations diet, milk had higher lipid concentrations than did the milk of rats fed a lower fat diet. MCFA were in lower concentration, but the amount of LCFA increased (Del Prado M et al. 1999). In contrast, in humans a low-fat diet increased the MCFA content in milk, as compared with a high-fat diet (Hachey et al. 1989). In both experiments, the low fat diet was supplemented with carbohydrates to equalize the total energy of the diets (Hachey et al. 1989, Del Prado M et al. 1999). In humans, consuming a high-carbohydrate diet increases the concentration of MCFA in milk (Harzer et al. 1984, Silber et al. 1988). In mice, the milk of dams fed sucrose long-term has an increased percentage of oleic acid (18:1) and a decreased percentage of linoleic acid (18:2), although the milk fat content was similar. However, the offspring of high-sucrose fed murine dams are heavier. They have an increased body fat, and the plasma levels of TG and glucose are higher (Ghusain-Choueiri et al 1995). The glucose level in the maternal diet is important in determining the sugar, lipid, and protein composition in milk. The mammary gland uses glucose as fuel in the biosynthesis of lactose and fatty acids. In rats, a diet with 24% glucose is required for optimal milk fat concentrations, whereas a diet with 12% glucose is sufficient to maintain normal milk lactose concentrations (Lanoue et al. 1994).

The properties and importance of milk are more complex than the sum of its components. Milk is not only nutritive, but also provides various bioactive compounds with roles in the ontogeny of the gastrointestinal tract. The bioactive agents from milk that modulate the gastrointestinal tract include hormones, growth factors, neuropeptides, anti-inflammatory agents and immunomodulators (Goldman 2000). These milk constituents are synthesized within the mammary gland, or are taken up by the mammary cells from the blood side and secreted into the milk. There are five major paths across mammary epithelium: the membrane, the Golgi apparatus, the milk fat globules, the transcytosis mechanism, and the paracellular route (Shennan and Peaker 2000).

There are many hormones in milk: adrenocorticotropic hormone, GC, thyroid stimulating hormone, thyroxine, growth hormone, prolactin, oxytocin, insulin and erythropoietin. The concentration of some hormones in milk exceeds the levels found in plasma (Grosvenor et al. 1992, Goldman 2000). The mechanisms for concentrating some hormones in milk secretion are not well known. They may be preferentially transferred in milk with the help of membrane receptors or binding proteins. For example, the normal mammary cells do not synthesize IGF-I, but synthesize and secrete insulin-like binding proteins (IGFBP)s, the carriers for IGFs. Thus, the higher concentration for IGF-I found in colostrum could be explained by the trapping of IGF-I by IGFBP (Grosvenor et al. 1992).

The growth factors in milk have been investigated intensively, because of their involvement in the ontogeny of the gastrointestinal tract. These are IGF-I and IGF-II, EGF, transforming growth factors α and β (TGF-α and TGF-β), lactoferrin and polyamines (Grosvenor et al. 1992, Goldman 2000). The growth factors are more abundant in colostrum than in the milk produced over the next several days. Both IGFs are present in colostrum. IGF-I is not synthesized by mammary cells, and flux studies indicate that IGF-I from blood contributes to the mammary secretion of this hormone. There is a higher concentration of IGFs in colostrum and milk than in mother's blood (Grosvenor et al. 1992). Although experiments with IGF-I proved to be more effective on intestinal proliferation and growth after systemic administration, oral administration of IGF also showed enhanced proliferation in the crypts (Xu et al. 1994).

EGF and TGF-α are distributed in all secretions, including milk. EGF and TGF-α peptides have an almost the same amino acid sequence, and both are synthesized as propeptides. Their precursors predominate in the mammary secretion, which suggests a local synthesis (Grosvenor et al. 1992). Oral EGF is a stimulus for epithelial cells growth *in vivo* (Berseth et al. 1987), and stimulates DNA synthesis in the intestinal mucosa (Puccio et al. 1988).

Lactoferrin has been shown to stimulate *in vitro* growth in cell cultures with fibroblasts or enterocytes, suggesting that it may be important in regulating the growth of intestine. Lactoferrin also facilitates iron absorption (Playford et al. 2000).

In addition to the their role as modulators of intestinal development, some milk growth factors such as IGF-I, EGF and lactoferrin have been found to increase nutrient uptake in suckling animals (Opleta-Madsen et al. 1991, Alexander et al. 1999, 2002). IGF-I administered orally in neonatal pigs (oral IGF-I in a dose of 3.5 mg/kg body weight/day for 4 days) enhances the rates of *in vitro* uptake for sodium (Na^+) and for Na^+-dependent absorption such as for D-glucose, L-alanine or glutamine (Alexander et al. 1999, 2002). In newborn rats, the oral administration of IGF-I was associated with BBM enzyme maturation (Ma and Xu 1997) and an increase in the intestinal protein content (Philipps et al.1997). Oral EGF administered to neonates precociously matures some intestinal functions. It increases the uptake of Na^+, Cl^- and H_2O (Opleta-Madsen et al. 1991), and reduces by 50% the macromolecule transfer across the intestine in suckling rat (Harada et al. 1990).

Neuropeptides found in milk, also known as brain-gut hormones, include somatostatin, neurotensin, gastrin, melatonin, bombesin and VIP (Grosvenor et al. 1992, Goldman 2000). The milk concentrations of VIP, bombesin, and neurotensin exceed their concentrations in mother's blood (Grosvenor et al. 1992). Other growth factors present in milk are fibroblast growth factor, platelet-derived growth factor (PDGF), and granulocyte colony stimulating factor (G-CSF) (Grosvenor et al. 1992, Calhoun et al. 1999, 2000). Anti-inflammatory agents in milk include antioxidants, enzymes, EGF and anti-inflammatory cytokines (Goldman 2000).

Immunomodulators in milk include antibodies, nucleotides, cytokines and leukocytes. All these constituents may influence positively or negatively upon the offspring's mechanisms of immunity. Also, they modulate the defense system in the early postnatal period. For example, milk is the main source of IgA antibodies and leukocytes during suckling which enhance the offspring's immune defense. Breast-fed children have a higher acceptance of maternal renal allografts, suggesting that breast milk decreases the reactivity of T cytotoxic cells to foreign tissue. Although breast milk may influence the baby's immune tolerance for foreign antigens, it is not known whether breast milk also influences immune tolerance for self-antigens (Goldman 2000).

The growth factors from milk interact with the gastrointestinal tract, and they may also be absorbed into the systemic circulation of the suckling animal (Goldman 2000). The immature gut is able to absorb macromolecules due to specific receptor-mediated transcytosis and nonspecific transcytosis (Jakoi et al. 1985, Pacha 2000). The immature intestine has higher permeability than does the adult intestine for both cellular and paracellular pathways (Wisser et al. 1978). Paracellular transport in the developing intestine has not been extensively studied, but transcellular transport during this period shows some interesting features: intestinal cells of altricial (immature hatched or born so as to require postnatal care) species contain a tubular system and endocytotic complexes until the time of weaning. All mammals contain this system until gut closure (Pacha 2000).

The maturation of the epithelium through the replacement of vacuolated enterocytes with nonvacuolated cells (Klein 1989), and the increase in proteolysis (Telemo et al. 1987), both lead to a decrease in macromolecule absorption. This process is called 'gut closure' and takes place in all species studied after birth, but the time when the macromolecular transport ceases is species dependent (Pacha 2000). In rats and rabbits, the gut closure takes place at weaning (Martin et al. 1997). Information about gut closure in human is still disputed. Sanderson and Walker (2000) stated that the human fetal intestine also contains an apical endocytic complex, which disappears after 22 weeks of gestation. Thus, it was suggested that gut closure in humans takes place at this time. Other authors showed that in breast-fed babies, the permeability of the intestine decreased faster than in artificially fed babies (Catassy et al. 1995). Moreover, postponing breast-feeding in newborn babies causes a delay in the normal decrease of intestinal permeability (Vukavic 1984). From the point of view of these authors, the gut closure in humans takes place at birth. Although human mothers give passive immunity transplacentally to the fetus, a reduced uptake of immunoglobulins is still detected in neonates (Sanderson and Walker 2000). The uptake of milk proteins is facilitated by the presence of colostral protease inhibitors in milk (Weström et al. 1985), as well as by specific receptors for some proteins such as IgG (Jakoi et al. 1985), EGF (Gonnella et al. 1987), and nerve growth factor (Siminoski et al. 1986). There is both direct and indirect experimental evidence that some hormones and macromolecules are transported intact across the intestinal mucosa during suckling, because plasma levels are increased of the recipients after oral administration (Table 4).

Macromolecules must adhere to the surface of the enterocytes before they are transported, and this adherence is greater in the developing intestine than in the adult intestine (Stern et al. 1984). In addition to the transcytosis through enterocytes, the ‘M’ cells (specialized epithelial cells of the follicle-associated epithelium) also contribute to this transport of macromolecules. The M cells are relatively low in number, and their capacity for transcytosis is not influenced by the maturation process. They represent weak points in the epithelium through which even very small amounts of proteins from food cross the intestinal epithelium (Kuch[illegible]rzik et al. 2000). Possible targets of these bioactive compounds from milk are the intestinal epithelium, enteric nervous system and mucosal immune system. Generally, these bioactive agents are degraded by intestinal proteolytic enzymes when they are orally ingested. Due to the special properties of milk, which contains protease inhibitors, these agents are active longer in suckling than in adult animals (Pacha 2000). For example, IGF-I and IGF-II in milk remain stable and active in the gastrointestinal tract for 30 minutes after oral administration to suckling rats (Fellah et al. 2001). Thus, milk-borne hormones and growth factors are delivered based on the properties of the immature intestine, and they may play a role in ontogeny of the gut (Pacha 2000).

Table 4. Milk-borne hormones and growth factors which are transported across the gastrointestinal tract experimentally

Milk-borne agent	**Route of administration**	**Result / Effect**	**Species**
Corticosterone	Orally administered	absorbed intact	adrenalectomized rats
Prostaglandins	Orally administered	absorbed intact	suckling rats
Insulin	Oral insulin	decrease blood glucose level ,	rats
Insulin	Oral insulin	have increased levels of insulin	calves
Prolactin	Orally administered	absorbed	suckling rat
EGF	Orally administered	absorbed	suckling rat
IGF	Orally administered	absorbed	piglets
TGF-β	Orally administered	absorbed	suckling mice
Thyroid-releasing hormone (TRH)	Injection to the mother	decrease the level of TSH	suckling rats
Thyroid-stimulating hormone (TSH)	Orally administered	increased serum T_4	suckling rats
Somatostatin	Orally administered	absorbed	suckling rat

Pacha 2000

It is unknown if the administration of a growth factor such as GLP-2 to the rat nursing mothers could affect the offspring. There is no experimental evidence that GLP-2 occurs in normal milk secretion or in milk after systemic administration to the lactating mother. GLP-2 belongs to the 'brain-gut hormone' class, from which some hormones have been shown to be secreted in milk (Grosvenor et al. 1992, Goldman 2000). These 'brain-gut hormones' were not expressed in mammary tissue when examined by the RT-PCR technique. These findings suggest that the 'brain-gut hormones' are actively concentrated in mammary gland from the general circulation (Chen et al. 1999). It could be speculated that GLP-2, which belongs to the same class of bioactive compounds, might be found in the milk of animals treated with GLP-2.

GLP-2 administration increases the intestinal uptake of carbohydrates. Cheeseman and colleagues (1997, 1998) found an augmentation of glucose transport across both the BBM and BLM in adult rats injected with GLP-2. Increasing the uptake of sugars from the intestine in dams could have a positive effect on the milk quality (Harzer et al. 1984, Silber et al. 1988) and on the nutritional status of the offspring such as (body weight, body fat, and plasma TG concentration) (Ghusain-Choueiri et al. 1995). Also, GLP-2, a recognized trophic substance for the intestine, increases the absorption of nutrients, and thereby improves the nutritional status in humans or animal models (Scott et al. 1998 and Jeppesen et al. 2001). Hence, GLP-2 treatment could influence positively the nutritional status of the lactating mother, and consequently her milk secretion. It may be speculated that GLP-2 will influence positively the amount and quality of the dam's milk, and consequently the offspring development, because it influences positively the nutritional status of the dams.

Drug passage from the blood of the lactating mother into her milk is mainly determined by the chemical and physical properties of the medication: its solubility in aqueous and lipid solution, the pKa, the molecular weight, and the binding to maternal plasma proteins. For example, drugs transferred by passive diffusion are readily found in milk; drugs which bind plasma proteins will reach the milk in very small concentrations (Feldman and Pickering 1986). GC are present in the milk of humans, cows, goats and rats (Grosvenor et al. 1992). The concentration of GC in milk is lower than in plasma, but the concentration can be increased during systemic administration of GC. For example, corticosterone in the lactating rat rapidly equilibrated between plasma and milk, so that variations in mother's blood concentration could be promptly reflected in milk (Angelucci et al. 1985). Corticoid binding globulin (CBG) proteins also have been found in human colostrum, as well as in the milk of rats or guinea pigs (Grosvenor et al. 1992). In nursing rats, labeled corticosterone appears in milk and in the neonatal plasma after suckling (Angelucci et al. 1985, Pacha 2000). In humans, doses of systemically administered GC must be minimized, because small quantities could go into milk. Thus, GC are in general contraindicated during breast feeding (Mutschler 1995). However, for prednisolone, the infant would ingest less than 0.1% of the amount administered to the mother (Ost et al. 1985). Because DEX does not bind to CBG, its diffusion is not affected by variations in the level of CBG. Thus, DEX is transferred into milk in a higher percent than other GC during nursing (Solomon et al. 2001). Therefore, DEX administered to nursing mother will be found in the dams' milk, and will be delivered to the offspring with the ingestion of her milk, thereby possibly influencing the development of the offspring.

1.3.3 Placenta and the ontogeny of the intestine

The placenta is an organ formed by the proliferating trophoblast to connect the mother and fetus. Despite its morphological simplicity, the placenta is a complex organ which accomplishes the materno-fetal transfer of nutrients to the fetus. It is also an endocrine organ as well as an immunologic barrier preventing the fetal allograft rejection (Pathology of the placenta 1978, Faber and Thorburn 1983, Ogata et al. 1997).

Nutrition

Materno-fetal transport of nutrients is thought to be largely passive, but there are various additional mechanisms contributing to this transfer, facilitated diffusion, active transport, bulk transport, pinocytosis and passage breaks in the placental villi. Strictly speaking, these breaks cannot be classified as a transfer mechanism, but they do permit the leakage of fetal blood into the maternal circulation (Pathology of the placenta 1978, Ogata et al. 1997).

Compounds that are transported to the fetus through the placenta include glucose, amino acids, fatty acids, vitamins, minerals and water. The placenta is also important for gas exchange: the transfer of oxygen from maternal blood to the fetus, and of carbon dioxide from the fetus to the mother (Pathology of the placenta 1978, Faber and Thorburn 1983, Ogata et al. 1997, Marconi 1997). Glucose is the principal metabolic fuel for the fetus and placenta. Under normal conditions, glucose is supplied by the mother, and only a small amount is recycled through the fetus back to the placenta (Hay 1988, Jones 1993, Marconi 1997). The placenta is a glucose-dependent tissue, mainly with anaerobic respiration converting glucose to lactate. Most of the placental lactate is transferred to the fetus. There is reduced utilization of glucose and lactate in the placenta, which reveals the role of placenta for supplying metabolites to the fetus rather than consumption for self maintenance (Hauguel-de Mouzon and Shafrir 2001). Amino acids are transferred across the placenta by an active as well as a passive mechanism. Metabolization of urea and ammonia occurs in the fetoplacental unit (Jones 1993, Meschia 1997). The essential amino acids are actively transferred. These are the branched chain and basic amino acids which include the essential amino acids (Young 1979). The branched chain amino acids are produced by the transamination of glutamate, which is an oxidative substrate in placental metabolism (Meschia 1997).

The placenta is relatively impermeable to polypeptides, although some maternal plasma proteins, such as antibodies, are transferred to the fetus by pinocytosis (Pathology of the placenta 1978, Faber and Thorburn 1983). Lipids, largely fatty acids, enter the placenta mainly by passive diffusion. However, a number of FABP have been identified in the syncytiotrophoblast, and are thought to facilitate the transfer of fatty acids. These include: FABPpm, FAT/CD36, FATP on the BBM and BLM of the syncytiotrophoblast, and L-FABP and heart-fatty acid binding protein (H-FABP) within the cytoplasm (Haggarty 2002). Some free fatty acids are reesterificated within the placenta. The newly synthesized or absorbed TG are hydrolyzed in the placenta, and fatty acids are released to the fetus (Hererra 2002). The lipoprotein lipase contained in the placenta allows the extraction of these fatty acids from the maternal acyl glycerols (Rothwell and Elphick 1982). Three TG-lipases have been identified in human and two in rat placenta (Hauguel-de Mouzon 2001). The transplacental gradient of long chain polyunsaturated fatty acids (LCPUFA) suggests that the placenta may play a role in the preferential transfer of LCPUFA (Haggarty 2002). From these fatty acids there is also a preference in placental transfer for docosahexanoic > α- linoleic > linoleic > oleic > arahidonic acid (Hererra 2002). The supply of the fetus with LCPUFA is critical for normal development in humans (Clandinin et al. 1980) because there is a low desaturation activity in the human fetus. In other species such as baboons, the fetus can synthesize LCPUFA from their precursors (Hererra 2002).

Transport of antibodies

The placenta is able to transport antibodies from the mother to the fetus, with some differences among species regarding this function. In rabbits and guinea pigs, the prenatal transfer of antibodies takes place through the yolk placenta. In humans and primates, the placenta transports antibodies through the trophoblast. In herbivorous mammalians, the antibodies are delivered after birth with milk through the small intestinal epithelium. For example, in sheep the newborn receives antibodies by ingestion of colostrum enriched with immunoglobulins. The antibodies from colostrum and later from milk are absorbed by the neonatal small intestine (Faber and Thorburn 1983) (see also page 36-37). Rats and mice have an intermediate position; the transfer of antibodies through the yolk sac is continued by postnatal transfer (Samuel and Steven 1979). In rats, antibody molecules injected into the maternal circulation or into the uterine lumen are transferred to the fetal circulation (Faber and Thorburn 1983). In humans, antibodies are given by the mother transplacentally to the fetus, although a reduced uptake of immunglobins is still detected in neonates from breast milk (Sanderson and Walker 2000).

Placental endocrinology

The placenta together with the fetus synthesizes steroids, progesterone, and estrogens, and this was the first example leading to the concept of the fetoplacental unit. Early in pregnancy progesterone is synthesized from cholesterol, and this synthesis increases with the duration of the pregnancy and with the size of the placenta (Jones 1993). The placenta produces large amounts of estrogens from fetus-supplied steroids. This conversion of the fetal precursors into estrogen is stimulated in humans by chorionic gonadotrophin (CG) (Barnea et al. 1986). CG secreted by the placenta maintains the steroid production in the mother and fetus (Jones 1993).

The placenta is also a rich source in prostanoids. For example, the levels of PGE_2 in the fetus and mother are correlated with the level of prostaglandin synthetase in the trophoblast. Prostanoids may regulate the pituitary-adrenal axis in the fetus (Jones 1993). The placenta produces placental lactogen (PL), which acts as an insulin agonist and has lipolytic activity in the mother. The effect on fetal or postnatal tissues in humans is small. PL indirectly enhances growth by increasing the mother's metabolism, and consequently the supply of nutrients (Cedard 1997). In contrast, ovine PL has an activity similar to growth hormone. In rats, ovine PL stimulates liver glycogen synthesis and fetal amino acid accumulation (Jones 1993).

A placental growth hormone, known as human placental growth hormone (hGH) is found in the blood of pregnant women. The physiologic role of hGH is not well defined, but there is a parallel between human placental levels of GH and IGF-I during pregnancy (Cedard 1997). The placenta metabolizes some maternal hormones such as thyroid hormones and catecholamines. The placental enzymatic breakdown of thyroid hormones and catecholamines provides a barrier to their transport to the fetus (Kaplan 1984). A reduction in uterine perfusion increases the permeability of the placental barrier. Thus, the catecholamines penetrate the placenta and regulate the fetal response to altered perfusion (Jones 1993).

The placenta is a target for growth factors and G-CSF has been demonstrated to cross the placenta (Calhoun et al. 2001). Although there is no evidence that other growth factors cross the placenta, several experiments suggest that some growth factors enable indirectly growth (Milner et al. 1996, Fant 1996). For example, the placenta has receptors for IGFs, insulin, EGF, TGFs and other growth peptides (Jones 1993). IGF-I and IGF-II act on their receptors and regulate the proliferation, differentiation and metabolism of the trophoblast. The IGFBPs function as regulatory loops at the maternal-fetal interface. IGFBP-I on the 'fetal side' of the placenta is produced by the fetal liver, and it regulates IGF activity and transport on this side (Fant 1996). EGF influences placental growth, and its concentration in amniotic fluid is correlated with the placental weight. EGF stimulates the PL and CG production, and represents a signal from either the fetus or mother to the placenta (Jones 1993). The possibility of growth hormones to communicate through the placenta is suggested by the correlation between the levels of IGF-I in maternal plasma and the fetal birth weight (Hall et al. 1984). Many other growth factors and receptors for growth factors have been discovered in the placental tissue (Fant 1996). Therefore, the placenta produces specific and general growth factors with roles in placental nutrition or in fetal ontogeny (Jones 1993). There is no evidence for the presence of receptors in the placenta for GLP-1 and GLP-2.

Taking into account all the properties of the placenta, it may be possible that experimental treatment during pregnancy with other growth peptides such as GLP-2 might directly or indirectly influence the growth and development of the offspring. It is unlikely that GLP-2 affects the functions of the placenta or directly alters the development or growth of the placenta, but it is possible that the peptide crosses the placental barrier due to breaks in the placental villi (Pathology of the placenta 1978), or due to the peptide being transferred through the yolk sac (Faber and Thorburn 1983). Experimental infusion with GLP-2 in pig fetuses showed that GLP-2 could pass into the maternal circulation (Petersen et al. 2001). As a recognized trophic substance for the intestine, GLP-2 increases the absorption of nutrients, and thus improves the nutritional status in humans or animal models (Scott et al. 1998 and Jeppesen et al. 2001). Hence, GLP-2 treatment could potentially influence positively the development of the fetus..

Transfer of pharmacological agents across placenta

Drug transfer across the placenta is due to passive diffusion. Drugs rarely are taken up into the placenta by specialized transport processes. The amount of drug crossing the placenta depends on its concentration in the maternal blood. In the process of drug transfer, the placenta behaves like a leaky lipid membrane. Non-ionized water-soluble drugs cross lipid membranes by diffusion if their molecular weight is less than 100 kDa. Lipid-soluble compounds cross membranes by diffusion if their molecular weight is up to 1000 kDa (Reynold 1979). The placenta contains enzymes enabling the oxidative metabolism for some drugs. Placental drug metabolism has a small capacity and does not protect the fetus from the effects of drugs (Reynold 1979).

GC administered systemically in pharmacological doses diffuse freely across the placenta and have shown a significant concentration in the fetal compartment, as well as suppression of fetal cortisol (Munson 1995). Although the use of GC in pregnant women was not associated with an increased incidence of major fetal malformations (CPS 2003), some authors consider GC to be contraindicated during pregnancy (Mutschler 1995). According to Food and Drug Administration (USA), systemic GC are considered as ‘C’ pregnancy risk factors (studies in animal models revealed adverse effects on fetus) (Turkoski et al. 2002). Some GC such as prednisone and prednisolone when given in pharmacological doses are metabolized by the 11β-OH-dehydrogenase enzyme in the placenta. In treatments with low doses of GC, this enzyme inactivates the drug, and only low concentrations of GC reach the fetus (Ehrich et al. 1972, Blanford et al. 1977, Reece et al. 1995). DEX is not reliably inactivated in the placenta, and is not recommended during pregnancy (Blanford et al. 1977, Reece et al. 1995). GC are bound in the blood to the CBG. This binding is reversible, and an increase in binding of GC to CBG decreases the plasma concentration of free GC, and consequently its biological activity (Myles and Daly, 1974). During the fetal period and postnatally until rodents reach the weaning time, there is an increase in the amount of CBG. This rise in CBG decreases the level of free GC and its biological activity, but increases its half-life (Leeper et al. 1988).

DEX passes through the placenta and doesn’t bind to CBG. The administration of DEX to the mother yields the same circulating levels of GC in the fetus in different developmental stages (Solomon et al. 2001). If a systemically active GC is administered during the fetal period, the intestine shows precocious maturation with an increase of digestive functions. For example, the administration of DEX in rabbit fetuses increases the intestinal nutrient uptake and the activity of BBM lactase and maltase enzymes (Buchmiller et al. 1994, Guo et al. 1995). DEX induces a catabolic state (Thomas et al. 1992), explained by a down-regulation of the hepatic IGF-I system (Kritsch et al. 2002). In young rodents, prenatal and postnatal DEX induces growth retardation in the offspring (Rooman et al. 1999, Okajima et al. 2001).

Little is known about the effect of GC on placenta. The cells producing PL decrease their number and secretion when the cortisol concentration rises in the fetus. This event is normally programmed before birth, and the decrease in PL switches the maternal metabolism for the demands of lactation (Ward et al. 2002). In the species in which this hormone plays a role in fetal development, such as ruminants and rodents, it may be speculated that DEX treatment before birth will affect the offspring by decreasing PL level (Jones 1993, Ward et al. 2002). Another mechanism of effect of DEX to interfere with fetal development is the placental IGF-I system, because it is known that DEX down-regulates the IGF-I system in the whole body (Kritsch et al. 2002).

1.3.4 Intestinal bacterial flora in intestinal ontogeny

Intestinal colonization with bacteria may play an ontogenic and regulatory role. The development of the intestinal microflora begins at birth when the neonate becomes exposed to the environmental bacteria. The bacteria colonizing the newborn intestine are acquired largely from the mother. Almost 400 bacterial species inhabit the human intestinal tract (Adlerberth et al. 2000). The groups of intestinal bacteria are represented by the aerobic and facultatively anaerobic bacteria (Enterobacteria, Enterococci, and Staphylococci), as well as the anaerobic bacteria (Bifidobacteria, Bacteroides, Lactobacilli, Clostridia, and others). The ratio of aerobic to anaerobic bacteria is 1/1 in the first postnatal week. The number of anaerobic bacteria increases from birth to adult life, reaching a ratio of 500/1 (Adlerberth et al. 2000).

Although the major groups of bacteria are almost the same in different human subjects, the strain and the proportion of these bacteria in the intestine is unique in each individual. Analyses of the chromosomal digest of bacteria from intestinal microflora using RT-PCR coupled with denaturing gradient gel electrophoresis show the uniqueness of the genetic fingerprints (Tannock 2002). Breastfeeding influences the pattern of the intestinal bacterial ecosystem (Adlerberth et al. 2000). Numerous studies were performed in humans to find differences in intestinal microflora, and their possible implications between breast fed and bottle fed infants. It is not established which of the factors found in breast milk determine these variations in the intestinal bacterial flora. However, the specific intestinal microflora and lower levels of casein in beast milk contribute to the low intestinal pH (Adlerberth et al. 2000). A low pH in addition to the milk factors may reduce the colonization with other Enterobacteria than Escherichia coli (E. coli) and strains of E. coli with high virulence (Bullen et al. 1977).

The presence of intestinal microflora is an important factor in colonization resistance for new bacterial stains. Epidemiological studies showed that the features of the intestinal microflora in breast-fed babies give protection against the development diarrhea, necrotizing enterocolitis, urinary tract infections, and septicemia (Hanson et al. 2002). The relatively simple flora of infants is not so effective in suppressing potentially pathogenic bacteria, but breast milk is effective by decreasing the ability of bacteria to cause disease. IgA secreted in milk blocks toxins and the attachment of bacteria to the mucosa (Hanson et al. 2002).

The intestinal microflora is a stimulus for the intestinal immune system. The intestine of animals with normal intestinal flora has ten times more IgA producing cells than do germ-free animals. The intestinal epithelial cells (IEC) are in contact with lymphoid cells, T cells, dendritic cells and polymorphonuclear lymphocytes. Different pathways transport antigens and bacterial products modulating the inflammatory response (Hershberg et al. 2002). The microflora may translocate through the intestinal epithelium, and the direct contact between the bacteria and the lymphoid system triggers immune responses, including the production of secretory IgA (Sanderson and Walker 2000).

Specialized epithelial cells of the follicle-associated epithelium, the 'M' cells, contribute to this transport of antigens. The M cells represent weak points in the epithelium resulting in enhanced transcytosis (Kucharzik et al. 2000). IEC express on the BBM toll-like receptors which recognize components of bacteria and induce proinflammatory responses in the intestine (Hershberg et al. 2002). Experiments with germ-free mice show that normal colonization of the mammalian intestine during neonatal life maintains the physiological steady state of the inflammation in the gut throughout later life (Cebra 1999). The balance of inflammation is maintained by the thymus-dependent lymphocytes, also termed helper lymphocyte, the non-circulating Th1 cells and the long-lived recirculating Th2 cells. The microflora is important in promoting Th1 mechanisms. The dominance of Th2 over Th1 immune responses in reaction to environmental antigens increases the incidence of atopic disorders such as food allergy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and allergic urticaria (Tannock 2002). A normal bacterial flora in the gut induces food tolerance to food antigens (Sudo et al. 1997). For example, the lipolysacharide from the membrane of E. coli and other enterobacteria enhances food tolerance (Kim et al. 1995). The mechanisms of these interactions are not known. It is possible that certain types of bacteria in the neonatal intestine facilitate the development of tolerance to harmless antigens. A high incidence of allergy in Western countries may be linked to the improper colonization of the intestine of these infants (Sanderson and Walker 2000).

Experiments with germ-free rats revealed the link between the gut flora and the enterocytes proliferation. Dietary fiber stimulates crypt cell proliferation, but it is the breakdown of fiber by the colonic microflora that is responsible for these proliferative actions. The fermentation of fiber leads to the production of short fatty acids which stimulate blood flow in the intestine, and enhance the release of GLP-2 and peptide YY. There is also a decrease in the number of enteroendocrine cells in the small intestine of germ-free animals (McCullough et al. 1998).

During postnatal development, the intestinal BBM modifies the patterns of protein glycosylation because of a change in fucosyl- and sialyl-transferases. At the time of weaning, fucosyl-transferase activity increases and sialyl-transferase activity decreases in the small intestine (Henning et al. 1994). There is a close parallel between sialyation and endocytic activity of the small intestine. The ability of the immature intestine to take up macromolecules is related to the presence of sialic acid residues on the BBM (Gill et al. 1999). Both GC and the gut microflora precociously induce these changes (Henning et al. 1994, Freitas et al. 2002, Nanthakumar et al. 2003). Indeed, these experimental results revealed that the microflora plays a role in the maturation process of intestinal epithelium (Nanthakumar et al. 2003).

1.4 INTESTINAL ADAPTATION

The capacity of intestinal absorption during suckling is altered by the same factors influencing the adaptation mechanisms during adult life: 1) the surface area of the intestine; 2) the proliferation and differentiation of enterocytes; 3) the fluidity of the BBM; 4) the abundance and activity of the nutrient transporters; and 5) the permeability of the BBM (Pacha 2000).

Intestinal adaptation is a biological process in which the intestine alters functionally and/or structurally in response to external or internal stimuli. The mechanisms involved in intestinal adaptation are still not completely understood. The adaptive changes of the intestine, which occur in response to the environment, enhance the survival potential and usually are helpful for the health of the host. For instance, the dietary components are important stimuli for intestinal adaptation (Thomson et al. 1986, Sanderson and Naik 2000) and the intestine must be able to adapt quickly to alterations in dietary content or load (Sanderson and Naik 2000). Many models of intestinal adaptation have been studied: fasting and malnutrition (Ferarris 1997, Ferraris and Carrey 2000), chronic ingestion of ethanol (Thomson et al. 1984), abdominal radiation (Thomson et al. 1983), and intestinal resection (Robinson et al. 1982, Park et al. 1994). The adaptation of intestinal absorption has also been examined in various physiological states: pregnancy (Musacchia and Hartner 1970), lactation (Cripps and Williams 1975), high carbohydrate loads (Sanderson and Naik, 2000), aging (Thomson 1980, 1981), and experimental diabetes mellitus (Thomson and Wild 1997). In this later example, diabetes, intestinal adaptation may be harmful, leading to increased absorption of glucose and lipids, which accentuate the unwanted hyperglycemia and hyperlipidemia.

The adaptive process includes both a morphological and a functional component. The dynamics of the morphologic parameters are changed by the proliferation of enterocytes in the intestinal crypts and by the rates of enterocyte migration (Thomson et al. 1994). The morphological changes do not always explain the alterations in nutrient uptake (O'Connor et al. 1999). For example, the uptake of nutrients might be enhanced without any change in villous size (Thiesen et al, 2003). The kinetics of transport are changed by variations in the maximal transport rate (V_{max}), by the affinity of the carriers for substrates which are actively transported (reflected in the value of the Michaelis constant Km), and by the permeability of the intestine for substrates taken up passively (Thomson and Wild 1997). In most experimental models of intestinal adaptation, enhanced uptake of actively transported nutrients such as glucose is achieved by an increased value of the V_{max} (Diamond et al. 1984).

The simplest model of intestinal adaptation, which is the response of the intestine to its dietary content, reveals changes in villous height, lipid composition of the BBM, alterations in the activity of BBM enzymes, and nutrient uptake (Thomson and Rajotte 1983, Keelan et al. 1990, and Daveloose et al. 1993). Dietary carbohydrate induces an intestinal adaptive response by increasing the hexose transporters to facilitate a higher rate of sugar absorption (Diamond et al. 1984). For example, adult animals fed a glucose-enriched diet have an increase in glucose uptake, as a consequence of the up-regulation of glucose transporters in the BBM and BLM (Cheeseman and Maenz 1998). Animals fed a fructose-enriched diet have an increase in fructose uptake and increased expression of the BBM fructose transporter, GLUT5 (Monteiro and Ferraris 1997). The intestinal adaptive process in response to the changes in dietary glucose is initiated in the intestinal crypts (Ferarris and Diamond 1992, 1993). The intestine may adapt by increasing the crypt cell turnover, the enterocyte migration rate, and the capacity of the transporters in the crypt cells to match a higher glucose load (Ferraris and Diamond 1992). Dietary lipids also influence absorption through specific adaptive mechanisms. Experiments performed with rats fed isocaloric semisyntetic diets enriched with SFA or PUFA show alterations in intestinal uptake of actively and passively transported nutrients. For example, feeding SFA increases the intestinal permeability to lipids, and increases the V_{max} for glucose uptake (Thomson et al. 1987). The intestinal adaptation which occurs in response to changes in dietary lipids can be used in our advantage: the malabsorption which occur with chronic ethanol intake or in response to abdominal radiation can be prevented by feeding SFA (Thomson et al. 1989, 1991), and the unwanted hyperabsorption of glucose and lipids which occurs with diabetes can be returned towards normal by feeding PUFA (Thomson et al. 1987).

Most lipid transport across the BBM is the result of passive diffusion and depends on BBM permeability (Hamilton 1998). For example, the jejunum is more permeable than the ileum and hence lipid absorption is higher in the proximal intestine (Meddings 1988, Shiau 1987). Similarly, the increased permeability of the intestine in suckling compared with adult rats enables an increase in fat absorption at time when the animal is consuming a high-fat diet, milk (Frost et al. 1983, Flores et al. 1989). The decrease in BBM permeability which occurs in aging is due to changes in the membrane lipid constituents (Swarz et al. 1985, Hubner et al. 1988, Keelan 1990). Dietary fatty acids are modulators of the BBM phospholipid fatty acid (Keelan 1990). In contrast, dietary cholesterol does not alter the BBM cholesterol composition, showing that cholesterol level in the BBM is stable (Keelan et al. 1994). Interestingly, changes in BBM fluidity may alter other the permeation of other molecules and the conformation of the binding sites on active transporters and thereby their activity (Meddings et al. 1990, Alberts et al. 1994). Thus, dietary lipids may influence sugar uptake rates.

A high protein diet increases amino acid uptake in rats (Sharrer 1972, Wolfram and Sharrer 1984). In mice, a high protein diet stimulates an increase of 77-81% in non-essential amino acid uptake, and a 32-61% increase in the uptake of essential amino acids absorption (Karasov et al. 1987).

Thus, the intestine of young animals is also capable of adaptation: feeding young growing rabbits for two weeks with a high cholesterol diet enhances the jejunal uptake of nutrients. The absorption changes may persist, and some further alterations appear when animals are switched from a cholesterol-enriched diet back to chow. When rabbits fed the high-cholesterol diet for 10 days were switched back to chow diet for 18 days, an enhancement in the jejunal uptake for glucose, galactose and leucine compared to chow fed group was detected (Thomson and al. 1987).

In addition, the dietary composition is a significant modulator for the structure and function of the intestine in young ages (Ferraris and Diamond 1989). Changes in the dietary content of carbohydrates, lipids, cholesterol and proteins during suckling and weaning influence the morphologic and/or uptake alterations of the intestine (Karasov et al. 1985, Thomson et al. 1987, 1989 and Keelan et al. 1990). Also, the previous dietary experience of the animal may influence the manner in which the intestine adapts to a dietary challenge at a later time. For example, if animals are fed in adulthood with a type of diet to which they have already been exposed during suckling or weaning, the resulting adaptive changes are different from those resulted from exposing these adult animals to a diet for the first time (Karasov et al. 1985). Late effects were found in experiments with rats after previous feeding with diets enriched with SFA or PUFA. One group of rats were weaned on PUFA for 2 weeks, switched to SFA for 8 weeks, and then switched once more to PUFA for 2 weeks. The second group of rats were weaned with SFA for 10 weeks and switched to PUFA for the last 2 weeks. A reduction in glucose absorption in the ileum was found in the first group compared to the second group (Thomson et al. 1989).

The adaptability of the intestine is also influenced by the nature of the diet provided to the pregnant or lactating dams: Jarocka-Cyrta et al. (1998) demonstrated that a change in the mother's diet at the time of birth and continued during suckling modifies the uptake in the intestine of the offspring. For instance, continuously feeding the mothers with a n-6/n-3 diet during pregnancy and lactation resulted in an increased uptake of glucose in the sucklings. The glucose uptake was compared with other groups of offspring whose mothers received a low n-6/n-3 diet, arachidonic acid diet, or docosahexaenoic acid diet. This evidence of a developmental window during the fetal, suckling, and weaning periods leads to the concept of "critical period programming" which enables an irreversible alteration of some biological mechanisms for the rest of life. Hence, the ontogeny programming is not entirely predetermined, and there may be effects of stimuli introduced during the ontogenic period, as reflected by the late effects of early nutrition (Karasov et al. 1985, Pacha 2000).

1.5 INSULIN-LIKE GROWTH FACTORS

The ontogeny of the gut is a result of a complex interaction between the mucosa, mesenchyme, growth mediators and the enteric nervous system (Pacha 2000, Henning et al. 1994). A number of peptides and hormones play a role in the development of the small bowel. Beside the well-known role of GC and thyroid hormones, other polypeptide growth factors are involved in the growth and maturation events of the small intestine (Pacha 2000). These growth factors include a number of proteins: insulin-like growth factors (IGF-I, IGF-II), epidermal growth factors (EGF), transforming growth factors (TGFα, TGFβ), PDGF, colony-stimulating factors (CSF) such as G-CSF, hepatocyte growth factor, fibroblast growth factor, and some interleukins (IL)s (Lee et al. 1993, Calhoun et al. 1999, 2001, Sukhotnik et al. 2003). These components are generally strong promoters of cell proliferation, and are released by epithelial cells, mesenchymal cells, or immune system cells. These substances act in an endocrine manner directly on the enterocytes, or in an autocrine-paracrine manner through interactions with other cells (Pacha 2000, Henning et al. 1994).

Growth hormone (GH) has a pivotal role in somatic growth. The physiological effects of GH depend on the action of somatomedins, which are polypeptide factors secreted by the liver and other tissues in response to GH. Initially designated as sulphatation factors, IGFs are the somatomedins found in humans. The IGFs, IGF-I and IGF-II, have been associated with cell proliferation, growth, and metabolic processes (Daughaday et al. 1972, Daughaday 2000, Rotwein 1991, Cohen and Rosenfeld 1995). Experiments with rat cartilage incubated with normal rat serum or GH showed that GH alone was inactive. This led to the hypothesis that GH acts through somatomedins (Daughaday et al. 1972, Daughaday 2000). Much of the local effect of GH is mediated by IGF-I, but in condrocytes GH may directly stimulate mitogenesis (Daughaday 2000). IGFs are single chain polypeptides related to insulin, and are synthesized in the fetal and adult liver, bone cells and other tissues. They are at least partially under GH control, and their presence stimulates DNA synthesis and cell differentiation (Rotwein 1991).

IGF-I promotes growth mainly in the postnatal period (Froesch et al. 1985). The IGF-I serum concentration is relatively low in the fetal stage, and increases after birth (Daughaday and Salmon 1997). IGF-II is an essential factor during embryogenesis and the fetal period. IGF-II mRNA and protein levels are high in most fetal tissue of all organs which have been examined including muscle, skin, liver, heart and intestine (Brown et al. 1986, Han et al. 1998). The level of IGF-II remains high after birth in humans, while it fells in rodent tissues. In rats, IGF-II mRNA was expressed at higher levels in the fetus than after birth (Holthuizen et al. 1997). In some tissues such as muscle, liver, heart and intestine, the high levels of IGF-II mRNA were maintained 3 weeks after birth (Brown et al. 1986), and then decreased until postnatal day 28 (Wallen et al. 1994, Hogg et al. 1994).

The biological activities of IGFs are controlled by their interaction with large soluble molecules, IGFBP. The IGFBPs facilitate the transport of IGFs in the body, serve as protection against IGF degradation, and mediate positively or negatively the interaction between IGFs and their cell receptors (Firth et al. 2002, Guck and Boisclair 1997). The IGFBP family consists of six proteins: IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6. All six proteins have been well studied, their expression and regulation have been described, their proteins purified, and their cDNAs and genes have been cloned and characterized in several mammalian species (human, mouse, rat, and cow) (Hwa et al. 1999). The major IGF transport function can be attributed to IGFBP-3. The most abundant circulating IGFBP in postnatal serum, IGFBP-3, is synthesized by liver and other tissues (Firth et al. 2002, Cohen and Rosenfeld 1995).

The IGFs act through specific cell surface receptors, IGF receptors (IGF-R) type I and type II, as well as the insulin receptor. IGF-I binds IGF-IR with high affinity, IGF-IIR with lower affinity, and insulin receptors with the lowest affinity. IGF-II binds only IGF-IIR. The insulin receptor can bind both IGFs, but with lower affinity than insulin (Cohen and Rosenfeld 1995). The role of IGFs in proliferation is mediated mainly by the IGF-IR. The metabolic actions of IGFs are mediated by their interaction with the insulin receptor (Oh et al. 1993). In addition to its role in cellular signaling (Nishimoto et al. 1987), IGF-IIR may be involved in the degradation of IGF-II, thus regulating the levels of IGF-II (Nissley 1997). The type I IGF-R is a heterotetramer, and has tyrosine-kinase motifs on its intracellular domains (Kelly et al. 1995). These kinases appear to phosphorylate a cytoplasmatic protein which is responsible for many of the biological effects of IGFs and insulin, 'the insulin-responsive substrate' (Nissley et al. 1991).

Both IGFs have been shown to be essential for normal embryonic and fetal development (Milner and Gluckman 1996). IGFs are also present in mammalian milk, which suggests that they may have a role in gastrointestinal development. Orally ingested IGFs are degradated by intestinal proteolytic enzymes, but the degradation activity in neonatal rat intestine is smaller than in adult rats. Colostrum is known to act as a protease inhibitor in the first nursing days (Pacha 2000). Milk also contains other compounds with protease inhibiting activities. For example, in human milk were found inter-alpha-trypsin inhibitor, alpha 2-antiplasmin, alpha 2-macroglobulin, antithrombin III, and antileukoprotease (Lindberg et al. 1982). Due to the protease inhibitors, IGF-I and IGF-II in milk remain stable and active in the gastrointestinal tract for 30 minutes after oral administration to suckling rats (Fellah et al. 2001).

IGF-I

Because IGF-I has a role in cell division for some cell types and mediates the GH effects, its therapeutic possibilities have been investigated intensely in the last several years. IGF-I starts acting in late gestation, whereas IGF-II may play a role earlier in gestation. IGFs do not cross the placenta, but the maternal IGF-I system indirectly influences fetal growth (Milner and Gluckman 1996). The placenta contains surface receptors for IGF-I, IGF-II, insulin receptors and hybrid receptors. These hybrids are formed from insulin, IGF and atypical receptors. Through these receptors, IGF-I and IGF-II regulate the proliferation, differentiation and metabolism of the trophoblast (Fant 1996).

The development and growth of an organism involves two processes: proliferation by increasing the number of cells, and growth in the cell size. The proliferation of a cell population depends on the balance between cell division and cell death. IGF-I controls the size of a cell population in four different ways: 1) mitogenicity inducer *in vivo* and *in vitro*; 2) promoter of growth in the size of the cell; 3) an anti-apoptotic role; and 4) helper factor for differentiation and transformation of cells (Baserga et al. 1997). In cell replication, IGF-I acts later in the G_1 phase to move forward the cell to the S phase, thus being a ‘progression’ factor (Warburton and Skinner 1996).

By changing the cell membrane permeability, IGF-I increases the uptake of nucleotides and stimulates kinases such as thymidine kinase. In addition, IGF-I increases the uptake of small molecules such as amino acids in cultured trophoblast of human placenta (Karl 1995). Sometimes this peptide promotes an increase in cell size but not cell division. In this situation, the cells are stimulated to enter into the S phase, but not to divide (Reiss et al. 1997).

IGF-I is one of the most powerful inhibitors of apoptosis. Therefore, targeting IGF-IR might be one of the treatments for tumors or their metastases (Rinisland et al.

1997). IGF-I is crucial for the transformation of a variety of oncogenes, and interestingly, oncogenes up-regulate the IGF system (Gelfant 1977).

IGF-I effect on the intestine

The intestine is a major target for IGF-I and GH. Generally, IGF-I and GH act on the intestine in an endocrine manner, but IGF-I is also expressed locally within the bowel and may be GH-dependent or -independent. The intestinal physiology is influenced by its own secreted IGF-I, as well as by circulating IGF-I. An important question is how to distinguish between the influences of locally secreted IGF-I and circulating IGF-I on the intestine (Lund 1997). The circulating concentration of IGF-I decreases during fasting and increases with feeding. Changes in jejunal mass during fasting and after refeeding are correlated with the serum IGF-I and with jejunal IGF-I mRNA (Winesett et al. 1995). IGF-I, but not GH, reverses the total parenteral nutrition (TPN)-associated atrophy of the intestinal mucosa in rats (Peterson 1997). GH has smaller effects on mucosal growth as compared with IGF-I, although IGF-I mediates the actions of GH on the intestine (Lund 1997).

IGF-I delivered subcutaneously following a 70% jejuno-ileal resection attenuated nitrogen and fat malabsorption in rats (Lemmey et al. 1994). Daily fat and nitrogen balance was calculated as the difference between the intake and the excretion of fat and nitrogen into urine and feces (Lemmey et al. 1991, 1994). This positive balance for fat and nitrogen suggests that the absorption of amino acids was enhanced by IGF-I in resected rats. IGF-I also increased total gut weight by up to 21% (Lemmey et al. 1994). The locally produced IGF-I also contributes to intestinal adaptive growth, both in fasting-refeeding experiments (Gelfant 1977) and in bowel resection studies (Mantell et al. 1995). The IGF-I receptor is increased in the jejunum and colon after small intestine resection. Resection also increased circulating IGFBPs. IGF-I treatment had no effect on IGF-I mRNA or IGF-I receptor density, but increased IGFBP5 in the jejunum. This increase in IGFBP5 is correlated with jejunal growth after IGF-I treatment (Gillingham et al. 2001). In some experimental models of inflammatory bowel disease (IBD), IGF-I administration attenuated inflammation (Howarth et al. 1998) and accelerated intestinal healing (Chen et al. 1997). However, IGF-I therapy in IBD is still under evaluation, because IGF-I could theoretically also promote excessive responses of the extracellular matrix and lead to fibrosis (Lawrance et al. 2001).

IGF-I administered orally to neonatal pigs (oral IGF-I in a dose of 3.5 mg/kg body weight/day for 4 days) enhances the rates of *in vitro* uptake for Na^+ and for Na^+-dependent absorption such as D-glucose, L-alanine or glutamine (Alexander et al. 1999, 2002). This effect is independent of changes in the mucosa mass or intestinal surface area. Oral IGF-I had no effect on intestinal growth, probably due to its short duration of administration, 4 days (Alexander et al. 1999, 2002). In contrast, systemically administered of IGF-I increased intestinal mucosa weight, villous high, protein content and proliferation (Alexander et al. 1999). Orally administered IGF-I did not change the BBM disaccharide activities, fructose uptake or glucose transporter abundance after 4 days of treatment in piglets (Alexander et al. 2001). The activity of Na^+-K^+-ATPase was greater for the group of rats treated with IGF-I, and this could explain the up-regulation of the Na^+ -dependent uptake (Alexander et al. 1999, 2001, 2002).

There are some controversies about the effects of IGF-I on BBM enzymes in neonatal animals. In experiments with newborn rats, Ma (1997) found that IGF-I administered orally as 1µg/day for 3 days increased the activity of lactase (LPH), sucrase and maltase. In newborn piglets, oral IGF-I increased the posttranslational processing of pro-LPH in BBM, but did not alter the activity of the LPH (Burrin et al. 2001). These studies suggest that milk-borne IGF-I may play a role in postnatal development of the gut.

The gastrointestinal tract is one of the most sensitive targets to IGF-I in the catabolic state induced by GC. The gut is the first to respond to IGF-I in this situation (Read et al. 1992). Subcutaneously administered IGF-I in rats protects against the catabolic effects of GC treatment. The weight decreases in the heart, liver, spleen, or intestine resulting from GC treatment are restored to normal by the administration of IGF-I (Thomas et al. 1992, Read et al. 1992, Kritsch et al. 2000). Moreover, the whole body catabolism induced by GC was explained by a down-regulation of the IGF-I axis (Kritsch et al. 2002). These data suggest that IGF-I could be used as an anti-catabolic agent in catabolic states such as those associated with the administration of GC (Thomas et al. 1992, Kritsch et al. 2002).

Therapeutic potential of IGF-I in intestinal diseases

The beneficial actions of IGF-I on the intestine (such as increasing the intestinal mucosal weight, villous height, protein content, proliferation, and uptake of glucose and some amino acids (Alexander et al. 1999, 2002) suggest that it could be used for therapy for infants with compromised intestinal function although IGF-I has not been studied in this setting. IGF-I also enhances the intestinal adaptive growth after bowel resection (Lemmey et al. 1994), and therefore might possibly be used in the treatment of patients with short bowel syndrome.

Human GH has been used as therapy for GH-deficient patients for the last 40 years. In the "GH insensitivity syndrome", which is characterized by structurally or functionally abnormal GH receptor, IGF-I is a useful treatment (Vaccarello 1995). IGF-I treatment must be carefully monitored because its systemic administration has effects in all tissues, and side effects such as hypoglycemia, headache and hypokalemia were reported with long-term IGF-I therapy (Abbasi and Rudman, 1995).

1.6 EPIDERMAL GROWTH FACTOR

EGF, a small polypeptide with 53 amino acids, was isolated in 1962 from murine submaxillary glands. EGF is involved in epidermal and epithelial cell proliferation *in vivo* and *in vitro* (Oberg et al. 1990, Mroczkowski et al. 1990). EGF and EGF receptors (EGFR) have been found in many tissues, and EGF is present in most fluids or secretions of mammals including milk (Fisher et al. 1990). EGF is secreted as a large precursor molecule (prepro-EGF) by many tissues such as kidney, intestine, thyroid gland, pancreas, and submandibular glands (Poulsen et al. 1986). Interestingly, the prepro-EGF mRNA that was detected in the distal tubules of the kidney is not processed to EGF. This suggests that prepro-EGF may have other roles in the kidney besides being a growth factor precursor (Mroczkowski et al. 1990). EGF may have a role in embryogenesis and fetal growth, since EGFRs are found in fetal tissues. EGF given *in vivo* induces precocious eyelid opening and tooth eruption. However, blocking the activity of EGF in pregnant rodents does not result in any major adverse effects (Gresik et al. 1998).

Maternal EGF does not cross the placenta, but EGF and TGFα may act on placental receptors (Jones 1993, Fant 1996, Cedard 1997). The concentration of EGF in the amniotic fluid is correlated with placental weight. Hence, EGF may represent a signal from either the fetus or the mother to the placenta. EGF also stimulates the placental secretion of human placental lactogen (hPL) and human chorionic gonadotropin (hCG), which are implicated in placental endocrinology (Jones 1993).

TGFα and EGF are structurally and functionally related. EGF and TGFα share the same surface cell receptor, namely the EGFR, and both have similar biological effects (Lee 1993). During fetal development, the level of TGFα is higher than that of EGF. This suggests that EGF substitutes for the role played by TGFα during the fetal and suckling period (Fisher et al. 1990, Dvorak et al. 1998). For example, in the fetal gastrointestinal tract the TGFα mRNA is easily detected, but the EGF mRNA can be detected only through polymerase chain reaction analysis (Miettinen 1993).

EGFR is a transmembrane glycoprotein with an extracellular ligand binding domain and an intracellular tyrosine kinase domain. The biological responses to EGFR activation are mediated by protein phosphorylation and stimulation of inositol metabolism through the phospholipase C pathway (Oberg et al. 1990). After ligand binding to EGFR, the complex becomes internalized and degraded. Rapid and late biological events are seen until the complex is completely degraded. The rapid effects are an increase in the transport of ions such as Ca^{2+}, Na^{+} and H^{+}, as well as an enhancement of inositol phosphate formation (Oberg et al. 1990, Scimeca et al. 1989). EGF increases the activity of the Na^{+}/H^{+} exchanger (NHE) in cultured cells (Scimeca et al. 1989), as well as in kidney or jejunal BBM vesicles (Barisic et al. 2002, Ghisan et al. 1992). EGF also induces slow responses such as stimulation of protein, RNA and DNA synthesis (Carpenter et al. 1976, Malo et al. 1982).

EGF acts as a 'progression factor' in cell mitogenesis, because it operates in early G_1 and promotes progression to the S phase (Warburton and Skinner 1996). EGF acts synergistically with GH in the proliferation process by activating a protein kinase that catalyzes phosphorylation of 22k GH. EGF also stimulates GH secretion in normal pituitary cells (Martin 1985).

EGF effects on the intestine

During the fetal period, EGF influences the formation of the hard palate, digestive tract mucosa and lung surfactant. At this early developmental stage, the peptide also promotes epidermal growth, maturation and differentiation of the gastrointestinal mucosa (Martin 1985). The mRNAs of EGF and TGFα are highly expressed in the intestine of suckling rats (Dvorak et al. 1998). The EGFRs are present postnatally along the entire length of the small intestine, and their number increases continuously until they reach adult values. Binding studies with [125I]EGF on mouse small intestine demonstrate this increase; and a maximum binding appears on the 21st day, which coincides with the weaning time (Gallo-Payet et al. 1987). EGF is secreted and produced by mammary glands, and the milk-borne EGF is a protective factor in breast milk (Dvorak et al. 1998). The increase in EGF concentration and EGFR number postnatally, and the presence of this peptide in milk, suggest a role for EGF in the development of the gut.

During late fetal development and the suckling period, EGF stimulates intestinal maturation (Beaulieu et al. 1981, 1985). EGF administration reduces by 50% the macromolecule transfer across the intestine of suckling rats (Harada et al. 1990). EGF increases the proliferation of the neonatal intestine, and also increases the activity of ornithine decarboxilase (ODC). ODC is involved in the metabolism of polyamines, which could also initiate gut proliferation (Goodlad and Wright 1989). In the mature intestine EGF stimulates cytoprotection, proliferation and migration of the cells, and induction of mucosal enzymes (Wong et al. 1999).

EGF increases the intestinal uptake of Na^+, Cl^-, water and glucose in mature (Opleta-Madsen et al. 1991, Hardin et al. 1996, Rongione et al. 2001) or suckling animals (Opleta-Madsen et al. 1991). This increase in glucose uptake after EGF treatment results from a translocation of the SGLT1 transporter from an internal microsomal pool into the BBM, as well as from recruiting more enterocytes in the glucose transporting population (Chung et al. 2002). EGF acts through protein kinase C (PKC) and phosphoinositol-3 kinase (PI-3k) (Millar et al. 2002), and increases the BBM area by recruiting additional microvillus membrane (Hardin et al. 1993, 1999).

After massive small intestinal resection, endogenous EGF is increased in saliva and is decreased in urine. EGF stimulates the intestinal adaptation occurring after intestinal resection (Thompson et al. 1999). After resection, the BBM area and the total absorptive surface increase until day 10. Postoperatively EGF treatment induces a further increase in BBM area (Chung et al. 2002). The EGFR redistributes from the BLM to BBM after small bowel resection, but there is no change in the total EGFR amount (Avissar et al. 2000). EGFR is predominantly located on enterocyte BLM, and as a consequence opinions are divided as to whether EGF has an effect when administered orally rather than systemically (Wong et al. 1999).

Therapeutic potential of EGF in intestinal diseases

EGF may be used as a therapeutic agent in several clinical situations. It has a protective role on the gastric mucosa (Tarnawski and Jones 1998, Jones et al. 1999). EGF is mainly involved during the process of healing of chronic ulcers, while TGFα acts after acute injury of the gastric mucosa (Jones et al. 1999). EGF also reduces mucosal damage and inflammation of the colonic mucosa in experimental colitis (Procaccino et al. 1994). In a rat model of necrotizing enterocolitis (NEC), EGF supplementation of the feeding formula reduced the incidence and severity of the disease (Dvorak et al. 2002). An interesting retrospective study of premature infants with NEC showed significantly diminished EGF concentrations in their serum and saliva. Monitoring EGF levels may distinguish infants at risk of NEC (Shin et al. 2000) and this suggests that EGF might be used to prevent or to treat NEC.

Blocking EGFR appears to be a promising strategy for the treatment of gastrointestinal neoplasia (Barnard et al. 2001). Blockade of EGFR signaling may reduce the growth in transformed epithelial cells, including the gastrointestinal mucosa. For example, *in vitro* and *in vivo* studies indicate that EGFR inhibitors reduce the growth of colorectal carcinoma (Mann et al. 2001). For example, trastuzumab is the first cancer therapeutic agent for breast cancer based on a monoclonal antibody against EGFR (Kirschbaum et al. 2000).

Both the fact that EGF enhances the uptake of water, Na^+ and glucose (Opleta-Madsen et al. 1991, Hardin et al. 1996, Rongione et al. 2001), and the increased proliferation in small bowel resection after EGF treatment (Wong et al. 1999, Chung et al. 2002), suggest that EGF may be used in the therapy of patients with reduced surface or function of the small intestine.

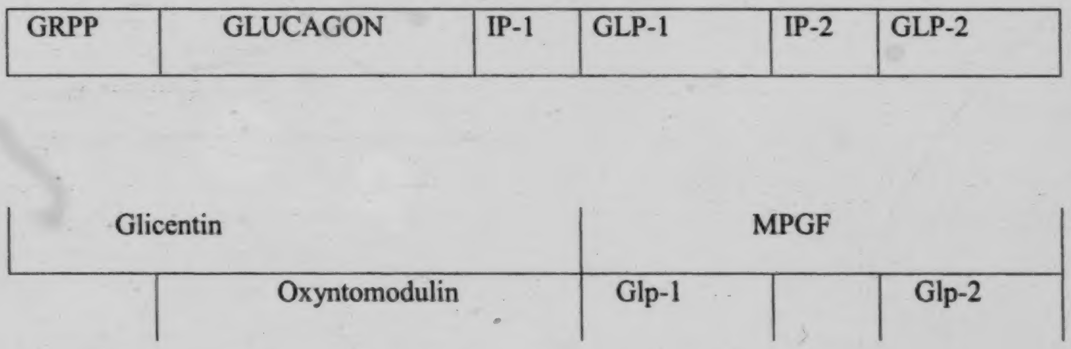
1.7 GLUCAGON LIKE PEPTIDE 2

Synthesis of GLP-2 and related peptides

Glucagon is a hormone secreted as the precursor proglucagon. The processing of this prohormone chain of amino acids results in glucagon and glucagon-related peptides (Figure 3).

Glucagon and glucagons-related peptides belong to a rapidly evolving family of peptides, namely the Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)/Glucagon Superfamily (Sherwood et al. 2000). In humans, the PACAP/glucagon superfamily includes nine hormones: PACAP, glucagon, glucagon-like peptide-1 (GLP-1), GLP-2, GH-releasing hormone, VIP, peptide histidine methionine, secretin, and glucose-dependent insulinotropic polypeptide (Sherwood et al. 2000).

Figure 3. Human proglucagon molecule and proglucagon derived peptides



Hartmann et al. 2000

GRPP: glicentin related pancreatic polypeptide; IP-1: intervening peptide 1; IP-2: intervening peptide 2; MPGF: major proglucagon fragment

These hormones are produced in the pancreas, gut, as well as in the central and peripheral nervous systems. They exhibit a variety of physiological roles. Some of them even act as neurotransmitters. The members of this superfamily are related by distribution (especially in the gut and brain), structure (homology from 21% to 48% with glucagon amino acids sequence), function (mainly activation of cyclic adenosine monophosphate synthesis [cAMP]), and receptors (there are seven transmembrane receptors) (Timothy et al. 1999).

The amino acid sequence of the preproglucagon genes is highly conserved among mammals. The products derived from proglucagon, glucagon and GLPs are highly conserved across animal species. The preproglucagon gene consists of six exons, E1 to E6, and five introns, IA to IE. Alternate splicing occurs for E4 and E5 exons in fish, but not in mammals (Timothy et al. 1999). Using statistical analyses of DNA sequence of preproglucagon genes from different animals, it was established that the glucagon superfamily genes resulted from duplication and amplification of the basic gene consisting of only four exons (Lopez et al. 1984).

Proglucagon is secreted by pancreatic α cells, intestinal L cells, and some neurons in the central nervous system. Posttranslational processing is highly specific, since these two types of cells give rise to specific glucagon-related peptides (Mojsov et al. 1986, Brubaker and Drucker 2002). Prohormone convertase generates glucagon and the major proglucagon fragment (MPGF) in pancreatic α cells (Drucker 2003). Glucagon, oxyntomodulin, glicentin, two intervening peptides, as well as GLP-1 and GLP-2 are processed by the endocrine L cells in the intestine (Drucker et al. 1996, Brubaker et al. 1997, Drucker 2003). In the brain, posttranslational processing liberates a profile of proglucagon-derived peptides, which overlap both the pancreatic and intestinal pattern (Drucker 2003). Chromatographic analyses of rat brainstem and hypothalamus revealed that proglucagon is preferentially processed to generate oxyntomodulin, glicentin, GLP-1, and GLP-2 (Larsen et al. 1997, Timothy et al. 1999).

Biochemistry

Molecular forms of GLP-2 (1-33) for human and rat are presented in following diagram.

Figure 4. The amino acids sequence for human and rat GLP-2:

Human: His^1-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp- (Arg)

Rat: His^1-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Thr-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp

Brubaker et al. 1997

Most GLP-2 is the result of the posttranslational processing of the proglucagon polypeptide in entero-endocrine 'L' cells of the gut. 'L' cells are located predominantly in the distal small intestine and colon. The release of GLP-2 is concomitant with the liberation of GLP-1, glicentin and oxyntomodulin from intestinal 'L' cells. The physiological role of glicentin and oxyntomodulin remains to be determined (Drucker et al. 2001). Parallel changes have been observed for GLP levels in rat blood (Fisher et al. 1997).

GLP-2 is present in three different forms in the circulation: bioactive GLP-2 (1-33), inactive GLP-2 (3-33), and inactive pancreatic MPGF. GLP-2 levels are usually measured using RIA (radioimmunoassay) or HPLC (high performance liquid chromatogragraphy) methods. The most common antiserum used in RIA procedures is antiserum UHTT-7. However, this antiserum also reacts with GLP-2 (1-33) and GLP-2 (3-33), and cross-reacts with GLP-2-containing molecules such as MPGF and proglugagon. The basal level of total immunoreactive GLP-2, GLP-2 (1-33), and GLP-2

(3-33) in the plasma of fasted rats was 700 ± 70 pg/ml. This level increases approximately 3.6 times after feeding the rats a chow diet (Brubaker et al. 1997). The primary stimulus for GLP-2 secretion is enteral nutrient intake, especially carbohydrates and LCFA (Fisher et al. 1997, Xiao et al. 1999, Burrin et al. 2001).

In humans, similar changes were observed for this peptide and derivates in response to a meal consisting of a coffee and a donut (~220 kcal). In this group of normal volunteers, plasma levels of immunoreactive GLP-2 increased two hours after the meal, from 851 ± 230 pg/ml to 1106 ± 211 pg/ml. Using a more specific binder which recognizes only the GLP-2 (1-33), namely the antiserum # 92160, the plasma level of full length GLP-2 (1-33) rises from 116 ± 22 pg/ml to 197 ± 98 pg/ml two hours postfeeding. Thus, intact GLP-2 accounts for 15 ± 4% of the total GLP-2 immunoreactivity in human plasma (Brubaker et al. 1997). No significant GLP-2 response was found after protein intake, but after mixed meals (proteins and lipids or proteins and carbohydrates), similar values for GLP-2 were obtained. The effect of proteins on the L cell remains to be investigated. A number of studies demonstrated that GLP-1 increases in response to a protein meal (Herrman et al. 1995), and that L cells show sensitivity to caloric loading (Burrin et al. 2001).

The short chain fatty acids (SCFA) resulting from the fermentation of non-absorbed dietary fiber stimulates the expression of proglucagon and the release of GLP-1 and GLP-2 (Burrin et al. 2001 and Reimer et al. 1996). Parenteral nutrition supplemented with SCFA, or the direct delivery of SCFA into the cecum, reduces the mucosal atrophy associated with total parenteral nutrition (Koruda et al. 1990).

Dietary fiber influences the small intestinal growth in diabetic rats. The increase of GLP-2 in blood matched the intestinal growth, suggesting that this growth may be mediated by GLP-2 (Thulsen et al. 1999). This experiment illustrates the importance of dietary fiber in the growth of the intestine. Dietary fiber or subsequent production of SCFA has an enterotrophic action, an effect that may be triggered by GLP-2 secretion (Thulsen et al. 1999).

Metabolism

Full length GLP-2 (1-33) is metabolized by aminopeptidase dipeptidyl peptidase IV (DPPIV) into GLP-2 (3-33). This process takes place in the intestinal epithelium. The cleavage limits the activity of the GLP-2 hormone (Drucker et al. 1997). The high levels of GLP-2 observed in patients with chronic renal failure suggest that part of the GLP-2 is eliminated by renal clearance (Brubaker et al. 1997). Full length GLP-2 (1-33) has a short half-life of 7 minutes, as compared with GLP-2 (3-33), which has a half-life of 27 minutes (Burrin et al. 2001).

GLP-2 Receptor

The membrane receptor of GLP-2 (GLP-2R) is a classic 'G' protein-coupled receptor (GPCR) with 7 transmembrane domains, and is 550 amino acids long. GLP-2R belongs to the GLP-1/glucagon/GIP receptor family, also known as the GPCR family B of receptors. Human GLP-2R is encoded in a single gene localized on chromosome 17p 13.3 (Munroe et al. 1999).

The location of GLP-2R and its pathways of action are still in dispute. There is evidence that GLP-2R may be situated in either the BBM or BLM of enterocytes (Thulsen et al. 2000), in the enteric neurons (Bjerknes et al. 2001), or in the enteroendocrine cells of the gut (Yusta et al. 2000). Thulesen et al. (2000) found GLP-2R even in enterocytes, predominantly in the upper part of the villi. In this study, rats were injected intravenously with radiolabeled GLP-2 [(125) I-GLP-2] and the binding sites in tissues were identified by autoradiography. An interesting finding was that (125) I-GLP-2 as well as GLP-2 metabolite (1-31) were displaced from the labeled places in the intestine by GLP-2 (1-33). The authors suggested that the metabolite, GLP-2 (1-31), may act on GLP-2R (Thulsen et al. 2000, Thulsen et al. 2002).

Bjerknes and Cheng (2001) demonstrated that GLP-2R is situated in the enteric neurons, rather than in the enterocytes. They used mice as an animal model, and GLP-2 receptor was investigated by reverse transcription polymerase chain reaction (RT-PCR) and *in situ hybridization* (ISH) methods. They showed that murine enteric neurons respond to a GLP-2 stimulus, and this may be an explanation of the inhibitory effect of GLP-2 on gastrointestinal motility. Also, GLP-2 enhances the c-fos gene in neurons and intestinal crypts, and the crypt response is neuron-dependent (Bjerknes and Cheng 2001).

Yusta and colleagues (2000) suggested that GLP-2R in humans is contained in a subpopulation of gastrointestinal endocrine cells. This hypothesis has been supported by Northern blotting, RT-PCR and immunocytochemistry methods. In this study, GLP-2R was not found in proliferating cells in the crypts or villous enterocytes, but instead GLP-2R was expressed on endocrine cells. This particular location of GLP-2R supports the existence of downstream mediators of GLP-2 actions. This finding and the variety of the GLP-2 effects support the hypothesis that GLP-2 acts in either a paracrine or an endocrine manner (Yusta et al. 2000).

The expression of GLP-2R in rat stomach, duodenum, jejunum, ileum, and colon was demonstrated using Northern blotting. Similar positive results are confirmed by the quantitative ribonuclease assay method, whereas protein expression is undetectable in seven other rat tissues including brain, heart, kidney, liver, lung, muscle and spleen (Munroe et al. 1999). Using RT-PCR, the most sensitive method, the mRNA of this receptor was found in rat brainstem and lung as well as the gastrointestinal tract, whereas protein expression was not found in heart, muscle and kidney (Yusta et al. 2000).

Brubaker and Drucker (2002) conclude that the GLP-2R receptor in the gastrointestinal tract has a restricted expression in enteroendocrine cells and enteric neurons. The density of GLP-2R mRNA in the gastrointestinal tract is low in stomach, reaches its highest levels in the proximal small intestine, and decreases towards the colon (Munroe et al. 1999).

Cellular mechanisms

All members of the family of B GPCRs increase the production of cAMP after their activation. GLP-2 administration to culture cells transfected with GLP-2R raised the level of cAMP four-fold (Munroe et al. 1999). Although Shen (1999) suggests that the GLP-2 proliferation is a result of the decreased cAMP levels, many other authors disagree; pointing out that the level of cyclic-AMP is increased by GLP-2R action both *in vitro* and *in vivo* (Yusta et al. 2000 and Lodish et al. 2000). The other downstream effectors of GLP-2R activation remain unknown.

There are different opinions regarding the pathways activated by GLP-2R and cAMP at the cellular level. The experiments performed with transfected receptor in baby hamster kidney fibroblasts (BHK) showed inhibition of apoptosis apparently mediated by cAMP, but not by PI-3K or mitogen-activated protein (MAP) kinase pathways (21). Delayed apoptosis may be attributable to the protein kinase A (PKA) pathway (Yusta et al. 2000). Jasleen and colleagues (2000) proposed that the GLP-2 proliferation of Caco-2 cells in culture is mediated by PI-3K kinase and by MAP kinase pathways.

New research data suggest that GLP-2 also acts at cellular level by an increase in the activity and amount of nitric oxide synthetase in the intestine. Tests were performed on TPN fed piglets after infusing 500 pmol/kg body weight/hour GLP-2 for four hours (Guan et al. 2003). Although it is not entirely clarified what are the cellular pathways activated by GLP-2 receptors, new experimental data imply that the intestinotrophic effects of GLP-2 are mediated by an increased blood flow and glucose utilization in the intestine in response to an increase in nitric oxide (Guan et al. 2003).

Physiologic effects

The main physiologic role attributed to GLP-2 is a trophic effect on the intestine. Indeed, the GLP-2 actions remain restricted to the gastrointestinal tract. The actions of GLP-2 are very specific compared to other peptides with trophic properties for the intestine, such as GH, EGF, IGF or IL-11. Also, GLP-2 is more potent than these other factors when comparing the gut mass after peptide treatment (Drucker et al. 1997). There is no evidence for cell proliferation or other effects on non-digestive tract tissues after three months of daily treatment with GLP-2 (Tsai et al. 1997).

Some of the GLP-2 actions occur immediately after administration (Wojdemann et al. 1998, Tang-Christensen et al. 2000, 2001) while other actions occur after hours or days (Cheeseman et al. 1996, 1998, Benjamin et al. 2000, Drucker et al. 1996, Burrin et al. 2000). Based on these differences in timing, on the location of the receptor (possible enteroendocrine), and on its various biological actions, it is assumed that GLP-2 exerts its potency in two ways: in a paracrine manner by unknown downstream mediators elaborated by entero-endocrine cells, and by an endocrine manner directly on the cells that express GLP-2R (Yusta et al. 2000, Drucker 2002).

GLP-2 has rapid actions (minutes) such as slowing the digestion and transit of food through the GI tract, increasing the absorption of nutrients, inhibiting gastric secretion and emptying (Wojdemann et al. 1998, Burrin 2001). GLP-2 enhances (hours) the hexose transport in intestinal BBM (Cheeseman et al. 1997) and BLM (Cheeseman et al. 1996, 1998). These actions may be mediated in an endocrine manner. GLP-2 also has slow actions (days) such as enhancing enterocytes proliferation, decreasing intestinal apoptosis (Drucker et al. 2001, Burrin et al. 2000), increasing the activity of sucrase-isomaltase and other BBM enzymes (Kitchen et al. 2000) as well as increasing the content of DNA and protein in small intestine (Litvak et al 1999, Chance et al. 2001), and reducing intestinal permeability of transcellular and paracellular pathways

(Benjamin et al. 2000). These slower reactions appear to be possibly mediated in a paracrine manner. Details of these physiologic and metabolic effects of GLP-2 are explained in the following sections.

Proliferation and growth

Numerous reports have correlated high serum levels of GLP-2 with hyperproliferation of the small bowel mucosa. For example, Drucker et al. (1996) demonstrated induction of epithelial proliferation by GLP-2. In their experiment mice were injected subcutaneously with 43.75µg of rat GLP-2, twice a day for 10 days. The mice demonstrated an increased small intestinal weight of about 50%. This experiment was performed with different amounts of glucagon-derived peptides, and even only 6.25µg rat GLP-2 given twice a day showed similar results (1.5-2 fold increase in small bowel weight). GLP-2 was the only peptide to produce increased mucosal thickness as a result of increased villus height, but not crypt depth or muscular thickness (Drucker et al. 1996). GLP-2 administration promotes crypt cell proliferation leading to the expansion of the intestinal mucosa. The GLP-2 treated enterocytes are longer and exhibit an increased number of microvilli.

The presence of enteral nutrients is not required for the trophic effects of GLP-2, since exogenous GLP-2 enhances the mass of the small bowel mucosa in rats maintained on TPN (Drucker 2003). In addition to the increase in intestinal weight, GLP-2 administration in adult animals for 10 days (1.75 mg/kg body weight twice a day) or for seven days (1 µg/h) enhances the content in proteins and DNA of the small intestine (Litvak et al. 1999, Chance et al. 2001). Interestingly, the experimental data from these tests suggest that proliferative effects of GLP-2 are augmented by co administrating the hormone neurotensine (Litvak et al. 1999).

Tsai et al. (1997) tested the bioactivity of GLP-2 *in vivo*. The efficacy of route, vehicle, dose, and frequency of administration was demonstrated in mice, and the results were analyzed after morphometric studies. When comparing the intraperitoneal, intramuscular and subcutaneous route of administration, it was observed that the subcutaneous route was more effective in inducing proliferation. The trophic properties of GLP-2 were slightly greater by its delivery with gelatin as compared to a saline vehicle. Intestinal proliferation occurred even after a single GLP-2 injection (Drucker et al. 1997, Tsai et al. 1997), and growth-promoting effects were achieved with a minimum of 0.1µg/g body weight/day, or with higher doses (Tsai et al. 1997). With regards to timing, a clear increase in growth and proliferation of the small intestine is obtained after 10 days of twice a day administration (Tsai et al. 1997).

GLP-2 increases the adaptive response to massive intestinal resection in rats (Scott et al. 1998). Administration of this peptide or its analogs was associated with an increase of the mucosal mass in the proximal jejunum and terminal ileum. In this study, Sprague-Dawley rats were divided into two groups: one group underwent a 75% mid-jejunum-ileum resection, while the other was a sham-operated group. In each group animals were given subcutaneously 0.1 µg/g body weight GLP-2 analog (protease resistant human GLP-2) twice a day or placebo, for 21 days. The groups were compared measuring the total weight of the rats, and the mucosal mass per centimeter length of intestine (Scott et al 1998). It is of interest that GLP-2 has also been used to enhance intestinal adaptation in humans with short bowel syndrome (see section Ontogeny page 88).

Apoptosis

It is unknown whether either enhanced proliferation or decreased apoptosis contribute more to the trophic role of GLP-2 (see section Proliferation page 80 and section Protection page 85). For example, GLP-2 treated mice exhibit both a decline in enterocyte apoptosis and an increase in enterocyte proliferation (Tsai et al. 1997, Drucker 2003). In premature pigs, the trophic effect of GLP-2 seems to be mediated mainly by the suppression of apoptosis, rather than by stimulation of cell proliferation. Premature pigs were fed with TNP and infused with human GLP-2 (25 nmol/kg body weight/day) for 6 days. Apoptosis was measured *in situ* by terminal dUTP nick-end labeling (TUNEL), and crypt cell proliferation was determined with the *in vivo* BrDU labeling procedure. In this study, GLP-2 was also found to increase intestinal protein accretion by suppressing proteolysis. Protein synthesis and degradation was calculated for intestinal segments (Burrin et al. 2000). Although anti-apoptotic properties of GLP-2 have not been directly proven in intestinal endocrine cells or enteric neurons, the activation of the GLP-2R receptor seems to stop the apoptotic pathways (Drucker 2003). Inhibition of apoptosis is demonstrated by *in vitro* and *in vivo* studies after the administration of GLP-2 and cytotoxic drugs, such as chemotherapeutic agents or nonsteroidal anti-inflammatory drugs (Boushey et al. 2001).

A recent study used mice treated with 5 flurouracil (5-FU) (400mg/kg body weight) or irinotecan hydrochloride -topoisomerase 1 inhibitor (280mg/kg body weight) for three days (Boushey et al 2001). A decrease in apoptosis and an improved survival of enterocytes was found in groups of mice treated with 10µg protease-resistant GLP-2 twice a day, for 3 days. Delayed apoptosis was the result of diminished activation of caspase-8 and caspase-3 (Boushey et al 2001). The prosurvival effects of GLP-2 administration were also observed *in vitro*. BHK cells transfected with rat GLP-2R and pretreated with protease resistant GLP-2 are protected from chemotherapy-induced apoptosis by decreasing the activation of the same pathways, caspase-3 and -8. Culture cells were pretreated with 20nM GLP-2 before the addition of 10µM irinotecan (Boushey et al. 2001).

The cellular mechanisms coupling cell survival to GLP-2R signaling are not understood. BHK cells transfected with GLP-2R showed that GLP-2R activation prevented apoptosis in a PKA-dependent and PI-3K-independent manner. Also, activated GLP-2R reduced mitochondrial cytochrome c efflux, one of the early steps of apoptosis (Yusta et al. 2002).

Enzymes

GLP-2 affects several functional aspects of the intestinal epithelium. GLP-2 enhances the activity of the following BBM enzymes: sucrase, maltase, lactase, glutamyl transpeptidase and DPPIV. The activity of the enzymes was measured by *in vitro* assay techniques where mice were injected subcutaneously with 2.5µg GLP-2 twice a day, for 8-10 days. The enhancement of enzyme activity found in GLP-2 treated mice occurred in parallel with intestinal growth. Hence, the total mucosal epithelium expressed normal enzyme concentrations (Brubaker et al. 1997). The mRNA of SI was also increased in the jejunum and ileum. In this experiment, Wistar rats received intravenously 40μg GLP-2 a day and were fed the TNP diet for 7 days (Kitchen et al. 2000). Although GLP-2 increased the absorption of ^{3}H leucine and C^{14}triolein (glyceryl triolein) in mice, no changes were found in the absorption of glucose or maltose. Nutrients were administered by gastric gavage followed by tail vein sampling for estimating the blood glucose concentration or ^{3}H and C^{14} levels (Brubaker et al. 1997). However, Cheeseman et al. (1996, 1998) have shown an enhancement of D-glucose transport in jejunal BLM after 2 hours and in jejunal BBM after 1 hour of GLP-2 intravenous infusions. In these studies, GLP-2 peptide (in a dose of 3.04 μg/hour diluted with saline) was infused through the jugular vein in rats, at a rate of 3ml/h for 2 hours. BLM vesicles were obtained after jejunal mucosal scraping, and ATPase activity was measured as a marker for purity of BLM. Preinjection of animals with an antibody binding both GLP-1 and GLP-2 blocks the increase of BLM glucose transport (Cheeseman et al. 1996). Cheeseman and colleagues (1997) explained the augmentation of glucose transport in the jejunum by an increased SGLT-1 abundance in BBM after GLP-2 administration. The glucose transport at BLM was also found to be increased, but GLUT-2 abundance was unchanged after GLP-2 infusion (Cheeseman et al. 1998).

The absorption of D-xylose is independent of bile salts, pancreatic exocrine excretion, or BBM enzyme activity. The amount of xylose absorbed in the gut and excreted in the urine is directly related to the mucosal absorptive surface and the intestinal transit time. The intestinal absorptive capacity for xylose in resected animals was restored after GLP-2 treatment. In this study (design described previously), Scott et al. (1998) also found an increase in intestinal mucosal mass after GLP-2 administration to either control or resected animals.

Permeability

GLP-2 is one of the factors involved in the maintenance of intestinal barrier function and mucosal integrity. Other factors include local and systemic growth factors, prostaglandins, endogenous bacterial flora, mucins and trefoil factors (Boushey et al. 1999). The property of GLP-2 hormone in reducing the intestinal permeability for both transcellular and paracellular pathways was demonstrated by Benjamin et al. (2000). Mice were injected with 5 µg of either GLP-2 or GLP-2 analog twice a day, for 10 days. Mice injected with saline served as the control group. The conductance of the jejunal tissue was measured with Na^+ and Cr-EDTA for the paracellular pathway, and with horseradish peroxidase for the transcellular pathway. This experiment proved that GLP-2 or GLP-2 analog increase the barrier function of the intestinal epithelium for both of these pathways (Benjamin et al. 2000).

Protection

GLP-2 is capable of protecting the intestinal mucosa from the damaging effect of chemotherapy (described in Apoptosis section) or the non-steroidal anti-inflammatory drug indomethacin (Boushey et al. 1999, 2001). In experiments with indomethacin treated-mice and concomitant administration of human GLP-2 analog, Boushey and colleagues found a decreased incidence of intestinal perforation, and bacterial septicemia. The study had two protocols using 7mg/kg or 20mg/kg indomethacin. Mice were pretreated 4 days with 2.5μg human GLP-2 analog twice a day before the indomethacin administration. Similar results were obtained whether GLP-2 analog was administered before, concomitant with, or after indomethacin (Boushey et al. 1999). Although these findings could result from an increased barrier function of the gut (Benjamin et al. 2000) and from decreased apoptosis of the enterocytes (Boushey et al. 2001), the major mechanisms of protection may be the reduced proteolytic activity (Burrin et al. 2000) and the alteration of intestinal cytokines (Boushey et al. 1999). Indomethacin enteritis is associated with an enhancement of tumor necrosis factor (TNF)-α, IL-2, and IL-10. The treatment with GLP-2 reduces TNF-α, IFN-γ, and IL-10 in the intestine. The myeloperoxidase (MPO) activity, an indicator of neutrophil infiltration, was also reduced after GLP-2 administration, but no link was found between this and the healing process (Boushey et al. 1999).

In a murine model of dextran sulfate-induced colitis, GLP-2 analog administration reduced mRNA of IL-1 cytokine (Drucker et al. 1999). The protective role of GLP-2 was explained in the case of indomethacin treatment by a decrease in apoptosis, and in the case of dextran sulfate-induced colitis by an increase in cell proliferation. Thus, the pro-proliferation and anti-apoptosis properties of GLP-2 play distinct roles in the protection of the intestine by this peptide (Drucker et al. 2000).

Regulation of genes

In a murine model, GLP-2 enhances the expression of the early responsive gene c-fos in enteric neurons and crypts (Bjerkness et al. 2001, Drucker et al. 2000). GLP-2R is present in the mesenteric nervous tissue and central nervous system, where GLP-2 induces the same c-fos protein (Tang-Christensen et al. 2000).

In vitro studies showed a direct relationship between the up-regulation of the SI gene and of CDX-2 protein. Also, GLP-2 enhances the expression of the sucrase-isomaltase gene *in vivo*. Thus, the CDX-2 gene expression was evaluated after GLP-2 treatment. Two groups of Wistar rats were fed with TPN and one group also received 40µg GLP-2 a day, for 7 days. The amount of SI enzyme was increased significantly in the ileum after GLP-2 treatment, but no changes were found in the level of mRNA for the homeobox protein CDX-2 (Kitchen et al. 2000).

Central nervous system

GLP-2R is found in the brain stem and in the dorsomedial hypothalamic nucleus. In the central nervous system, glucagon-derived peptides are produced only by neurons in the nucleus of the solitary tract and in the reticular nucleus. GLP-2R location in the brain stem has the distribution of proglugagon secreting neurons. There is a GLP-2 neuronal pathway that connects the nucleus of the solitary tract with the dorsomedial hypothalamic nucleus. GLP-2 serves as a neurotransmitter in this specific pathway, and inhibits food intake (Tang-Christensen et al. 2000, 2001). The discovery of GLP-2 and other peptides in the gut endocrine cells and also in the neurons of the central nervous system support Pearse's theory that both have a common neuroectodermal origin (Andrew 1982). Some endocrine cells, chemoreceptors, the brain, and the epithelia, have cells that form amine compounds. Pearse initiated the name of 'APUD' neuroendocrine system for these cells secreting polypeptide hormones [Amine

Precursor has been taken Up, the cell Decarboxylates it to form serotonin (5-HT) from 5-hydroxytryptophane or a catecholamine from dihydroxyphenylalanine (hence APUD)] (Pearse 1966, 1969). The APUD system secrets amines and peptides with various functions such as neurotransmitters, hormones, and modulators of neural action, thus it could be suggested that GLP-2 might play the same roles.

Ontogeny

GLP-2 may play a role in the regulation of intestinal growth and maturation during ontogeny, since GLP-2R is expressed in the fetal and neonatal rodent gut (Burrin et al. 2000 and Lovshin et al. 2000). Interestingly, the mRNA of GLP-2R is higher in fetal and neonatal rat intestine than in adults. This suggests a role played by GLP-2 in gut development, and/or in the transition from neonatal to adult intestine. However, the involvement of GLP-2 in fetal development still needs to be clarified, since the mutant proglucagon deficient mice (pax6 SEY^{NEU}), which has a deficiency in GLP-2 producing cells and less than 5% proglucagon mRNA transcripts, exhibit normal intestinal development. This implies that normal levels of GLP-2 are not critical for development in fetal murine intestine (Lovshin et al. 2000).

Using a pig model, Petersen and coworkers (2001) did not find any role for GLP-2 on intestinal growth in fetuses, despite the presence of GLP-2R. In this experiment fetal and neonatal pigs were TPN fed, and also received a 2 hour infusion with 12.5nmol/kg body weight human GLP-2, twice a day for 5-6 days. The treatment had no effect on gut growth in the fetuses, but increased the weight and villous height in neonates. The small intestine in pigs responds to the trophic effect of GLP-2 treatment only after birth. On the other hand, GLP-2 administration in pig fetuses increased the level and activity of aminopeptidase N mRNA. In neonatal pigs GLP-2 enhances aminopeptidase N and maltase mRNA levels and activities. These results suggest that the gut responsiveness to the intestinotrophic action of GLP-2 is associated with gut maturity (Lovshin et al. 2000, Petersen et al. 2001).

GLP-2 may be used in the treatment of patients with the short bowel syndrome, or in patients with reduced intestinal surface aria as a result of either inflammation (such as Crohn's disease) or injury (such as chemotherapy and radiation). The clinical experience in the treatment with GLP-2 is limited to a small number of patients without their ileum and colon, and consequently with impaired GLP-2 secretion. This study was done on patients with functional short bowel syndrome who underwent surgery in the previous 4-17 years. The cause of intestinal resection was Crohn's disease in 8 patients, ulcerative colitis and surgical complication in 6 patients, and ischemic infarction in one case. GLP-2 was administered subcutaneously in a dose of 400μg twice a day for 35 days. Balance studies were performed before and after treatment. Patients received identical diets with a fixed carbohydrate: fat: protein ratio during the two balance periods. They were unrestricted to salt and beverage intake, but were instructed to record any kind of intake. The balance studies were performed during 72 hours, and all oral intake and stomal output were weighed. Specific measurements were done for energy content using bomb calorimetry, carbohydrates using the Kjeldahl method, fat using gas-liquid chromatography, sodium and potassium using flame photometry, and urinary creatinine using the Jåffe method. Diet and fecal nitrogen were converted to weight of protein. The intestinal absorption of energy was improved from 49.9% to

53.4% and the absorption of carbohydrates from 69.7% to 74.1%. The effect of GLP-2 on fat and protein absorption was negligible. Also, the average gain in absorption of sodium and potassium did not reach positive results, but GLP-2 improved the total body weight. These results demonstrate that the treatment with GLP-2 improves the intestinal absorption and nutritional status in short bowel syndrome patients. These positive results raise the possibility of the extension of GLP-2 treatment to other gastrointestinal disease (Jeppesen et al. 2001).

In summary, GLP-2 acts as a trophic hormone with its effects restricted to the small intestine and it also plays a regulator role in the intestinal ontogeny.

1.8 GLUCOCORTICOSTEROIDS (GC)

Introduction

Corticoids are physiological hormones secreted by the adrenal glands. The adrenal steroids are produced in the following histological regions of the adrenal cortex: GC in zona fasciculata (first layer), mineralocorticoids in zona glomerulosa (second layer), and sex-steroids in zona reticularis (third layer). Corticoids are classified as GC and mineralocorticoids according to their physiological activities. If a corticosteroid has potency on the deposition of glycogen in the liver, it is called a GC (Goodman and Gilman's 1996). In humans, the naturally occurring GC is cortisol, whereas in mice and rats the main form is corticosterone (McDonald and Henning 1992). The release of cortisol or corticosterone is regulated by the hypothalamic-pituitary-adrenal (HPA) axis.

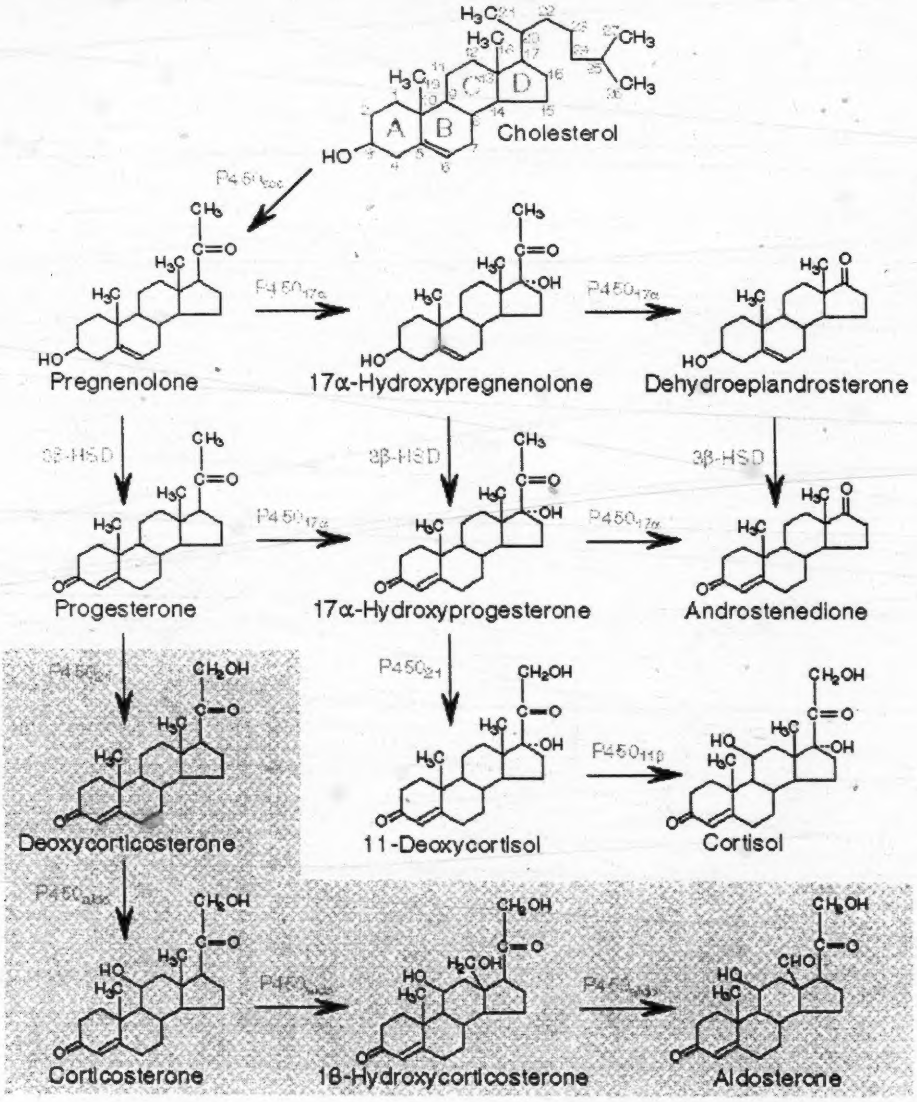
The hypothalamic corticotropin center initially releases corticotropin-releasing hormone (CRH), which stimulates the ACTH secretion from the pituitary gland. Pituitary ACTH release leads to the cortisol or corticosterone secretion from the adrenal cortex (William and Dluhy 1998). The HPA axis is an integrated system that maintains physiological levels of GC. Three mechanisms coordinate the HPA axis: the diurnal rhythm of steroidogenesis, the negative feedback by adrenal corticosteroid secretion, and the increase of steroid synthesis which occurs in response to stress.

The diurnal rhythm is induced by central neuronal centers and by the ACTH peak that occurs in the morning. Consequently, the GC level in the circulation rises at 8 a.m. in humans. In mice, the peak of corticosterone is observed between 4 p.m. and 10 p.m. (Coffigny et al. 1978). Negative feedback is the main mechanism involved in maintaining the proper GC blood level. The administration of synthetic GC blocks ACTH release. Stress can override the negative feedback control, and thus induce an increase in the circulating levels of all adrenal corticosteroids.

Biosynthesis

The biosynthesis of corticosteroids is called corticosteroidogenesis. This occurs in the mitochondria from cholesterol, either at rest or after ACTH stimulation (Stocco et al. 1996). Based on histological analyses, the adrenal cortex was separated into three zones, but functionally the adrenal cortex consists of two regions: the outer zona glomerulosa and the inner zona fasciculata/reticularis (Figure 5).

Figure 5. Pathways of corticosteroids biosynthesis


Cholesterol
A
B
C
D
P450scc
Pregnenolone
P450$_{17\alpha}$
17α-Hydroxypregnenolone
P450$_{17\alpha}$
Dehydroepiandrosterone
3β-HSD
Progesterone
17α-Hydroxyprogesterone
Androstenedione
P450$_{21}$
Deoxycorticosterone
11-Deoxycortisol
P450$_{11\beta}$
Cortisol
P450aldo
Corticosterone
18-Hydroxycorticosterone
Aldosterone

Goodman and Gilman's 1996

The pathways that are active only in zona glomerulosa are shown in dark-gray; those that are unique to zona fasciculata/glomerulosa are shown in gray.

Cells from the outer zone have receptors for angiotensin II and an enzyme that catalyzes mineralocorticoid production, called aldosterone synthase. Cells from the inner zone lack angiotensin II receptors, and have two enzymes that catalyze the glucocorticosteroids biosynthesis, namely 17α- hydroxylase and 11β-hydroxylase (Goodman and Gilman's 1996).

Transport

Cortisol in the blood is bound to the plasma globulin and albumin. The globulin is known as CBG. The CBG has low binding capacity but high affinity for cortisol, whereas albumin has high binding capacity but low affinity for cortisol (Myles and Daly 1974). More than 90% of all circulating cortisol is protein bound, but this binding is reversible. A chemical structure containing Δ4-3-ketone is essential for binding the corticosteroid to CBG. The new synthetic corticosteroids have substituent groups that decrease the binding to CBG, but increase the plasma concentration of free corticosteroid and consequently enhance their biological activity (Myles and Daly 1974).

Metabolism

The metabolism of GC occurs through: 1) reduction of the 4,5 double bound in the liver and other tissues; 2) reduction of the 3-ketone substituent to a 3-hydroxyl in the liver; 3) reversible oxidation of the 11-hydroxyl group in the liver and other tissues; 4) reduction of 20-ketone; and 5) oxidation of the 17-hydroxyl group (Haynes and Murad 1985). About 70% of the GC metabolism takes place in the liver (Sutherland

1970). In humans, there are also intestinal sites of GC metabolism, which are saturated before the hepatic sites (Barr et al. 1984). Most of the GC is excreted in the urine. The ring-A-metabolites are coupled in the liver or kidney with sulphate or glucuronic acid, resulting in sulphate esters or glucuronides. These compounds are excreted by the kidneys. Small amount of the GC can be detected in the fecal, biliary and breath CO_2 secretion (Haynes and Murad 1985). During pregnancy, GC use is relatively safe due to a placenta enzyme, the 11β-OH-dehydrogenase. In treatments with low doses of GC, this enzyme inactivates the hormone, and only low concentrations of GC reach the fetus (Ehrich et al. 1972, Reece et al. 1995). In humans, the cortisol half-life is about 1.5 hours. The GC metabolism is slowed by a 1, 2 double bond and by fluorination. These chemical features are the most often used in the production of GC compounds with a long half-life (Haynes and Murad, 1985). For example, DEX, due to its chemical features is not inactivated in the placenta and diffuses freely across the placenta resulting in relatively high concentrations in the fetus (Reece et al. 1995).

Glucocorticoid receptor

GC are considered to be lipids due to their high solubility in organic solvents. They diffuse across the plasma membrane and act inside the cell (Haynes and Larner, 1975). The receptors for corticosteroids are members of the steroid/thyroid/retinoid nuclear receptor family. Until recently, it was believed that there were only two corticosteroid receptors: the mineralocorticoid receptor (MR) or type I corticosteroid receptor, and the glucocorticoid receptor (GR) or type II corticosteroid receptor. The classic GR has high affinity for synthetic GC, whereas MR has high affinity for both corticosterone and aldosterone. Thus, MRs are unique because they have two distinct physiological ligands, depending on the cell and tissue in which they are expressed (Sheppard 2002). In addition to different corticosteroid receptor expression, tissues also express 11β-dehydrogenase (11βHSD), an enzyme involved in corticosteroids metabolism. There are two isoforms of 11βHSD: 11βHSD1 which increases the GC action by forming active GC from inactive metabolites, and 11βHSD2 which transforms GC into their inactive metabolites (Albiston et al. 1994). The aldosterone specificity on MR in tissues is conferred by the 11βHSD2 enzyme. This enzyme converts corticosterone and cortisol to their inactive metabolites, and thus enables aldosterone access to MR. In tissues expressing MR but not having 11βHSD, the higher endogenous GC than aldosterone circulating levels and the similar affinity of MR for these steroids result in GC binding on MR (De Kloet et al. 1993).

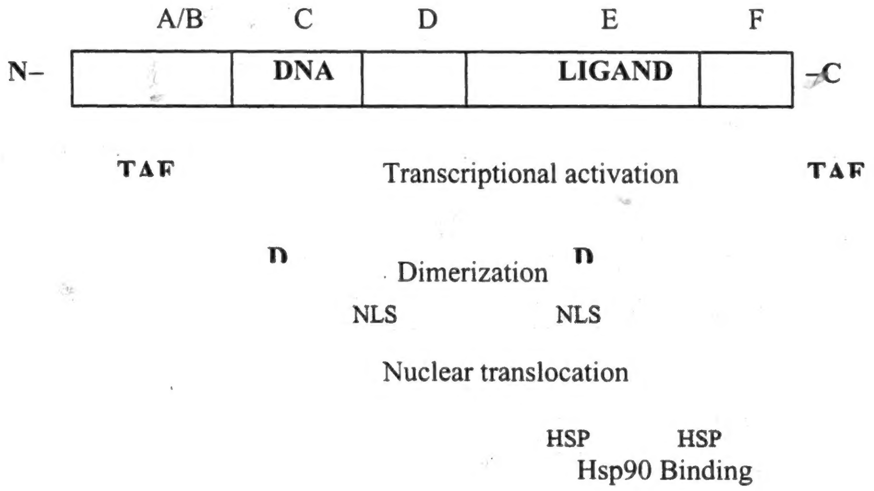
GR, MR and 11βHSD2, but not 11βHSD1 are expressed in the intestinal epithelial cells (Sheppard et al. 1999). The levels of their expression influence the effects of GC on the intestine. In the duodenum and jejunum, there is a low level of MR and 11βHSD2. Thus, GC rather than aldosterone regulate corticosteroid responses in these organs. In contrast, the ileum and colon contain GR, MR and 11βHSD2. Interestingly, if the endogenous secretion of GC is stopped, a synthetic GC but not aldosterone could restore ion transport in the colon. This suggests that GC and the interrelation among GR, MR and 11βHSD2 maintain the intestinal ionic transport (Sheppard 2002).

The human steroid/xenobiotic receptor (SXR) and the rodent ortholog pregnane X receptor, which are orphan corticosteroid receptors, have been identified in the intestine. Their activation by corticosteroids induces the expression of the MDR1 gene as well as cytochrome P450 3A (CYP3A) gene. The MDR1 gene encodes a transporter that protects cells by effluxing drugs, and the CYP3A gene is important in the metabolism of xenobiotics and steroids. The role of these new receptors in the intestine is yet to be determined (Sheppard 2002).

The glucocorticoid receptor investigated most is GR. GR exhibits a structure/function domain similar to the steroid/thyroid/retinoid nuclear receptor family. Each receptor consists of six regions A-F based on amino acid similarity (Figure 6) (Hache et al. 1999). The 'C' domain contains a zinc finger motif that constitutes the DNA-binding domain. An inhibitory protein complex binds to the hormone binding domain, and maintains the receptors in a transcriptionally inactive state. Their activation occurs through binding the ligand with the formation of the receptor-hormone complex (Lamarco et al. 1996).

The binding of the GC and the dissociation of the inhibitory protein complex change the receptor into a transcriptionally active form. The active receptor-hormone complex exposes the DNA binding site. The receptor-hormone complex then becomes capable of further diffusion into the nucleus of the cell, and binds to the nuclear chromatin (Haynes and Larner 1975, Bamberg et al. 1996). This mechanism accounts for the genome-mediated effects of GC. The role of the remaining protein complex is less clearly understood.

Figure 6. Schematic representation of functional motifs within glucocorticoid receptor


A/B
C
D
E
F
N–
DNA
LIGAND
–C
TAF
Transcriptional activation
TAF
D
Dimerization
D
NLS
NLS
Nuclear translocation
HSP
HSP
Hsp90 Binding

Hache et al. 1999

A/B variable amino-terminal region; C cystein rich central domain - DNA binding domain; D domain - variable hinge region; E domain - ligand binding domain; F domain; variable C – terminal region

The free GR exists in the cytoplasm as a multi-protein compound. This compound results from binding the 'pure' GR to an inhibitory protein complex. The multi-protein compound was also found to be linked to several kinases of the MAPK signaling system (Croxtall et al. 2000). The inhibitory protein complex contains the several heat shock proteins Hsp90 and Hsp70, immunophilins FKBP52, cyclophilin40, and protein p23 (Johnson et al. 1995, Pratt 1997). Hsp90 is the protein that assembles all these components into a protein complex, and connects this complex with the pure GR. The protein complex produced through Hsp90 is coupled to the intracellular protein kinases, a family of serine/threonine kinases including Src, Raf, and Mek components of the MAPK system. This family of kinases is also called Erks which stands for extracellular regulated kinase, because these kinases phosphorylate and activate the Erk pathway (Pratt 1997). The protein complex containing Hsp90 becomes a chaperone system within the cells. This system is involved in signal transduction for a variety of hormones and growth factors. The Hsp90-chaperone system may modulate the anti-proliferative effect of the GC through the Erk pathway (Pratt 1997, 1998).

Because Hsp90 exits as a dimer, and because the immunophilins are competing with each other for the binding sites, two different immunophilins [FKBP52, 52-kDa FK506-binding immunophilin (FKBP52) or cyclophilin40 can be bound to the same protein complex (Pratt 1998). This results in the existence of two different GC receptors. These different receptors, GRα and GRβ, coexist in cells (Bamberg et al. 1996). GRβs may antagonize the action of GRαs on the glucocorticoid responsive genes (Honda et al. 2000).

Cellular mechanisms of action

Genome mediated effects

In the nucleus, the receptor-hormone complexes bind glucocorticoid responsive elements as a dimer. Genes are activated only in the presence of the right combination of regulatory proteins. Each GC has a specific response, and each tissue has a different combination of gene regulatory proteins (Johnson 1987). In general, the GC nuclear reaction alters the synthesis of a specific mRNA, and consequently changes the protein synthesis (Bamberg et al. 1996). This reaction induces either positive (transactivation) or negative events (transrepression) by repressing the genes and/or alterating the mRNA (Krane 1993). Transactivation may be the result of a derepression mechanism (Godowsaki et al. 1987). The hormone-receptor binding increases the role of the functional enhancer-activating proteins, which are operational even in the absence of GR. This mechanism proposes that GRs unmask or derepress the enhancer-activation domain. Transrepression results from inhibition of different factors such as activator protein-1 (AP-1), nuclear factor kB (NFkB), and the signal transduction and activation of transcriptional family (Sandborn et al. 2000).

The response of the cell to GC implies two steps: an early effect involving the activation of early response genes, and a delayed effect activating the secondary response genes as a result of primary –response proteins (Bruce et al. 1994).

Rapid effects (non-genomic)

GC may act rapidly by a mechanism that inhibits the cytosolic phospholipase A_2 (PLA_2) activity, and consequently arachidonic acid (AA) release. These effects result from ligand occupation of GR and Hsp90 protein complex dissociation, but not by changes in gene transcription. The nuclear translocation of GR is not required for rapid inhibition of AA release (Croxtall et al. 2000, 2002). The rapid effects of GC have been revealed in cell culture experiments. These rapid effects of GC are mediated in cells via annexin1 (ANXA1), a protein on cellular membrane, ANXA1 is a well-characterized substrate for PKC. GC increase the phosphorylation and the amount of the ANXA1, and this is associated with the inhibition of cytosolic PLA_2 activity and AA release.

There is a competition between the phosphorylated ANXA1 and receptor binding domain SH2 of growth factor receptor bound protein (Grb2-SH2), because both share a similar N-terminal sequence. In its phosphorylated form ANXA1 displaces Grb2 from growth factor receptor complexes and blocks the JNK1 and MAPK/Erk kinases pathways, which are signals for cell proliferation. The inhibition of the Erk pathway blocks the translocation of PLA_2 to membranes, and subsequently the AA liberation (Croxtall et al. 2000, 2002). GC have rapid anti-proliferative actions that can be explained by the decrease in AA release, and consequently decrease prostaglandin synthesis and inhibit the signal pathways for growth factors (Croxtall et al. 2002). Experiments performed with cultured cells exposed to DEX confirmed the inhibition of EGF receptor signals and PLA_2 (Croxtall et al. 2000). The inhibition of growth signals has also been observed in cultured cells co-stimulated with hepatocyte growth factor (HGF) and DEX (Skouteris et al. 1996). Similar results have been obtained *in vivo*, were DEX blocks growth factor-induced signal transduction. For example, in rat liver exposed to DEX, insulin receptor kinase preferentially phosphorylates ANXA1. This might explain a part of the anti-insulin effects of GC (Karasik et al. 1988).

Permissive effect

The actions of some hormones are not observed unless the cells have been exposed to GC. This effect was called the 'permissive effect' of GC. Hormones enabled in their activity by GC include: cathecolamines, thyroid hormones, growth hormone and ACTH (Baxter 1979). GC induces the permissive actions mostly for hormones acting on GPCRs, and increase adenyl cyclase activity (Haynes and Murad 1985, Haynes and

Larner 1975, Baxter 1979). GLP-2R belongs to the family of GPCRs and is likely to be up-regulated by steroid hormones. Generally speaking, all GPCRs, are up-regulated by steroid hormones. In experiments with GPCRs exposed to GC treatment, an augmentation in the level of mRNA for these receptors was found. For example, β-adrenergic receptor mRNA levels increases threefold within two hours after DEX exposure (Morris and Malbon 1999). There is also experimental evidence that GC increases the adenyl cyclase signaling inside the cells (Meier 1997). (see chapter 8 Potential interaction between GC and GLP-2, page 114)

Glucocorticoid responses

The effects of GC are widespread throughout the body, because most of the tissues are direct targets for GC, and GC may directly or indirectly influence all tissues (Bruce et al. 1994). GC display a diversity of responses. These include an alteration of sugar and protein metabolism, an anti-inflammatory and an immunosuppressive effect due to modulation of the stress response and the immune system, a stimulation of the maturation processes, and an induction together with mineralocorticoids of the epithelial sodium transport. An important effect of GC is their anti-inflammatory action, acting on mast cells, macrophages, endothelial and fibroblastic cells. GC responses in inflammatory reactions include increasing the number of circulating neutrophils, decreasing the number of circulating lymphocytes and monocytes, inhibiting the accumulation of neutrophils and macrophages, and preventing the permeability rise in inflammatory sites. These suppressive inflammatory responses are the basis for GC use in the treatment of certain diseases, including some gastrointestinal disorders such as the inflammatory bowel diseases and autoimmune hepatitis (Baxter 1979). GC block the synthesis or the release of prostaglandins, leucotrienes, bradykinins and cytokines. They also interfere with complement activity, as well as the leukocytes and macrophages migration. These effects, especially for systemic-GC, take place not only in inflammatory sites, but also in all tissues. For this reason GC treatment may be potentially associated with adverse events beyond the area of the therapeutic target (Haynes and Larner 1975, Baxter 1979).

In mammals, GC in physiological doses have a trophic role in the development of all tissues during fetal period (Fowden 1995, Challis et al. 2001, Sheppard et al. 2002). The best known of these is the effect of GC on lung development (Bolt et al. 2001). Even normal GC doses improve the development of other organs such as the intestine. For example, experiments with adrenalectomized Sprague-Dawley rats reported effects such as atrophy of the intestinal epithelium, an increase in apoptosis, and a decrease in crypt proliferation (Foligne et al. 2001). The intestine has different reactions relative to the type of the GC, the duration of their administration, and the developmental stage of the animal when the GC are administered. For example, a 7 day treatment with prednisolone increases the absorptive capacities of the jejunum and ileum for galactose, without effects on the intestinal mucosa or cell kinetics (Batt and Peters 1976). The long-term administration (28 days) of prednisolone has the same stimulatory effect on absorption, but induces atrophy of the mucosa and inhibition of cell turnover. Similar results were obtained after 28 days of treatment with betamethasone instead of prednisolone (Batt and Scott 1982). The administration of prednisone for four weeks in weanling male rats had no effect of on D-glucose uptake and this may occur due to the younger age of the animals. If budesonide was given only for two weeks to these animals, the uptake of fructose was increased. Both prednisone and budesonide have no effect on the jejunal or ileal uptake of L-glucose or D-mannitol, which suggests that the passive paracellular contribution to sugar uptake is also unaffected by these steroids. Also, the lack of effect of either prednisone or budesonide on the value of the V_{max} of glucose uptake in these four week postweaning rats suggests that there was no change in the activity (i.e. the V_{max}) of the SGLT1 transporter. Giving prednisone, SGLT1 abundance is reduced in the jejunum of these animals, but this does not affect the activity of the transporter. This suggests that there is no association between the SGLT1 protein abundance and the transporter activity (Thiesen et al. 2003).

Glucocorticosteroids and intestinal permeability

The permeability of both the transcellular and paracellular pathways influences the total intestinal permeability. The adult intestine has a lower permeability than the immature intestine for both the cellular and paracellular pathways (Wisser et al. 1978). The transcellular transport in immature intestine is dependent on membrane permeability, and special systems enabling the transport of macromolecules. These include a tubular system and endocytotic complexes which both cease at the weaning time in rats, moment called 'gut closure'. GC treatment expedites the process of gut closure, and this decreases the absorption of macromolecules (Pacha 2000) (see subchapter 1.3.2 Milk and milk-borne hormones in ontogeny, page 30). There is an increased BBM fluidity, and consequently an increased BBM permeability in the jejunum and ileum of the suckling rats compared to adult animals. In the adult stage there is a decrease in fluidity explained by changes in BBM lipid composition. The amount of total lipids decreases with a parallel increase in protein content of the BBM, and the proportion of the phospholipids also increase with age. Membrane phospholipids composition changed with age, with a decrease in sphingomyelin and an increase in phophatidylcholine (Schwarz et al. 1985). GC administered to suckling rats or to pregnant rats caused an increase in the BBM fluidity of the suckling rats compared to the control group (Neu et al. 1986). Adult rats exposed to DEX show a similar increase in BBM fluidity due to a decrease in the membrane phospholipids content (Brasitus 1987). Moreover, Golgi membranes and liposomes exhibit a greater permeability after GC treatment (Dudeja et al. 1988). Because of these increases in membranes fluidity after GC treatment, it could be expected that GC also enables a greater absorption of compounds such as lipids to be taken up by diffusion. Measurements performed for lipid uptake generally show an increase after GC treatment, but results are variable implying the mechanism of lipid absorption is more complex (Thiesen et al. 2002).

Changes in BBM fluidity may alter not only diffusion of possibly absorbed molecules, but also the conformation of the binding sites on active transporters (Alberts et al. 1994) (see chapter 1.4 Intestinal adaptation, page 52). For example, by fluidizing villous-tip microvillus membrane vesicles, *in vitro*, a reduction in the Km of glucose transport was observed. These data suggest that the characteristics of the glucose transporter are dependent on its local membrane environment (Meddings et al. 1990).

The paracellular transport is also higher in immature than in adult intestine. GC stimulate this pathway of transport, as found in experiments with premature pigs

(Sangild et al. 1993). In rat adult intestine, DEX administration causes a significant decrease in the intestinal resistance (Spitz et al 1994). A decrease in transepithelial electrical resistance can result from a permeability alteration in either the trans- or para-cellular pathway, but analyses of fluxes for Na^+ and $[^3H]$ mannitol attributed this defect to the paracellular pathway. The rate-limiting barrier to diffusion via the paracellular space is the tight junction, and DEX decreases the resistance of the tight junction (Spitz et al 1994). Meddings and Swain (2000) obtained similar results with DEX treated rats, demonstrating an increase in intestinal permeability for mannitol and lactulose, suggesting that GC increase the permeation through the paracellular space.

Glucocorticoid effects on intestinal uptake

Generally speaking, short- or long-term GC administration increases monosaccharide absorption (Batt and Peters 1976, Batt and Scott 1982, Scott et al. 1981, Crake et al. 1984). For example, the *in vitro* uptake of D-galactose was enhanced in experiments with rats treated for 7 or 28 days with prednisolone. Giving betamethasone also increases the *in vitro* uptake of D-galactose, but with a parallel decrease in the enterocyte number (Batt and Peters 1976, Batt and Scott 1982, Scott et al. 1981). In humans with short-term administration of hydrocortisone, glucose absorption after jejunal perfusion with glucose was enhancedOne group received intravenous hydrocortisone and one group received intraluminal hydrocortisone, until both groups reached similar plasma cortisol levels. Hydrocortisone (100 mg/l) given by intraluminal perfusion increased sodium, water and glucose absorption, in contrast to intravenous hydrocortisone that had none of these effects (Crake et al. 1984), probably because the intravenous hydrocortisone is mostly acting on young enterocytes which have not emerged yet and not on mature ones. GC may cause different effects depending on which response predominates, the increase of the digestive-absorptive function or the decrease in the epithelial cell population (Henning et al. 1994, Scott et al. 1981). In the case of fructose, the response of the intestine to GC treatment is to increase uptake. This increase is not associated with an increase of the amount of GLUT5 protein or expression of mRNA. Probably the increase in fructose uptake observed with GC is due a post-translational control of GLUT5 (Thiesen et al. 2002, 2003).

Lipid uptake in the intestine (see Chapter 1) occurs mainly by passive permeation across the UWL and BBM, although the uptake of LCFA, cholesterol, and bile acids may also involve the fatty acid binding proteins in the BBM such as FABPm and FAT, as well as the cytosolic transporters, I-FABP, L-FABP, and ILBP (Schoeller et al. 1995). Prednisone increases cholesterol uptake in the jejunum as well as the ileal uptake of lauric (12:0), palmitic (16:0), and linoleic (18:2) acids. Treatment with budesonide increases the uptake of oleic acid (18:1) in the jejunum and of linoleic acid (18:2) in the ileum (Thiesen et al. 2002). Predisone increases the uptake of more fatty acids than does the locally acting budesonide, and this mechanism may be a result of the greater systemic effects of prednisone (Thiesen 2002).

The intestine adapts in response to physiological or pathological stimuli, including variations of the lipid composition of the diet (Thomson 1982). The type of lipids in the diet, SFA or PUFA, modulate the GC effects on intestinal uptake of lipids: in animals fed PUFA, prednisone enhances the jejunal uptake of 12:0, 18:1, 18:2 and ileal uptake of 18:3; budesonide enhances the jejunal uptake of 16:0, 18:1, 18:2 and the ileal uptake of 18:1. In animals fed SFA, prednisone has no effect in the jejunum and increases the uptake of 18:3 in the ileum; while budesonide treatment decreases uptake cholesterol in the jejunum and 16:0 in the ileum (Thiesen et al. 2002).

These variations in lipid uptake may be mediated by alterations in the lipid content of the BBM (Keelan et al. 1996). A change in the lipophilic properties of the BBM due to GC would be expected to change the uptake of all lipids, but this did not occur. The alterations in lipid uptake associated with GC treatment or dietary lipids are not associated with changes in mRNA levels for L-FABP and ILBP. Other lipid transporters in the BBM or cytosolic I-FABP were not assessed in this study (Thiesen et al. 2002).

Glucocorticoid effects on intestinal ontogeny

GC are one of the most important endocrine signals in the development and maturation of the intestine (Henning et al. 1987, 1994, Foligne et al. 2001). GC in physiological doses have a trophic effect on the growth of the intestine (Foligne et al. 2001). The major GC in the blood of rats is corticosterone (McDonald and Henning 1992). In rats, there is a surge of corticosterone before the time of weaning, which precedes the changes in BBM enzymes. The absence of GC did not stop the maturation process, but the rate of the maturation decreased significantly (Henning et al. 1994). The best known effect induced by GC on the immature intestine is the precocious maturation of intestinal structures and function (Henning et al. 1994, Sangild et al. 1995, Dai et al. 2002, Nanthakumar et al. 2003). For example, the activities of BBM sucrase and isomaltase in rats are observed after the 15th-16th day of life. The administration of exogenous GC during the first or second postnatal week causes precocious maturation of these enzymes (Henning et al. 1994). A synergistic effect on induction of SI enzyme was observed after concomitant treatment with GC and thyroxine (Leeper et al. 1998). DEX treatment in 10 day old rats causes a precocious appearance of SI activity and mRNA; on the 16th day, DEX produces an accelerated rise of SI activity and mRNA; after the 18th day, DEX has no effect on the activity or mRNA of SI (Nanthakumar and Henning 1993). In rats, there are three phases of GC responsiveness of the developing intestine: 1) an early phase (10th day) when GC induces gene activation; 2) a later response (≅16th day) where changes occur in the kinetics of the enterocytes; and 3) a period without GC responsiveness (Solomon et al. 2001). The early effects after GC administration may be caused by a GC-induced transcription of SI; the later effects may be induced by GC-induced changes in the crypt-villous kinetics (Nanthakumar and Henning 1993). The replacement of immature cells on the villi with more mature ones from the crypts after GC exposure reflects that mesenchymal cells facilitate the GC effects (Henning et al. 1994).

Trehalase mRNA, a marker of intestinal maturation in mice, is also increased after GC treatment (Solomon et al. 2001). The activity of the BBM LPH enzyme decreases and SI increases in the normal ontogeny of the intestine. The effect of GC on ontogeny of LPH is less clear, because both increases and decreases in the enzyme activity are reported after experiments with GC (Henning et al. 1994, McDonald and Henning 1992). The maturation of numerous enzymes is enhanced after GC administration, including salivary amylase, pepsinogen, pancreatic amylase, ileal hydrolases, intestinal pyruvate kinase, glutamine syntethase, hepatic ornithine aminotransferase and hepatic tryptophan oxygenase (Sarantos et al. 1994). These enzymes are not stimulated in intestinal culture cells unless mesenchymal factors are added to the culture medium (Kedinger et al. 1998, 2000). Mesenchymal elements are essential to induce maturation events in the intestinal mucosa (Simo et al. 1992, Henning et al. 1994, Kedinger et al. 1998, 2000). GC also induce maturation of gastrin receptors, ileal bile acid transport, small intestine pinocytosis, as well as intestinal absorption of immunoglobulins (Sarantos et al. 1994).

During the first three weeks of life, until rodents reach the age of weaning, there is an increase in plasma corticosterone which is parallel to an increase of CBG. These variations in CBG could affect the level of free GC and its half-life (Leeper et al. 1988). Numerous experiments have used DEX to study the effects of GC on the rodent intestine. DEX was the preferred GC, because it doesn't bind to CBG, and because administering DEX yields the same circulating blood levels in different developmental stages. DEX also passes through the placenta, and could be suitable for experiments with pregnant animals (Reece et al. 1995, Solomon et al. 2001). The doses of DEX used in previous studies vary from 0.0125 to 2.5 nmol/g of body weight, and have an effect on intestinal maturation (Solomon et al. 2001). Based on this and the experimental work of Park, the dose used in many experiments was 0.128 µg DEX/g body weight of rat, and administration of DEX was subcutaneous once a day (Park 1994, Thiesen 1996).

If systemically active GC are administered during the fetal period in rabbits, monosaccharide uptake is enhanced (Buchmiller et al. 1994). In mature rabbits treated with DEX, the *in vitro* uptake of glucose in the small intestine is also increased (Iannoli et al. 1998). The administration of DEX in rabbit fetuses increases the intestinal nutrient uptake, as well as enhances the activity of BBM LPH and maltase. Measurements of glucose and proline uptake were performed *in vitro* with the everted sleeve technique (Buchmiller et al. 1994). Similar results were obtained in a rabbit gastroschisis model treated with DEX. The lactase activity is increased 70% by DEX, but remains lower than that of a normal fetus. Maltase activity is also increased by DEX, and the rate of glucose absorption is increased 100%, but remains lower than in the normal fetus (Guo et al. 1995).

In rodent models, DEX administration during suckling causes functional maturation events, such as an increase in the activity of BBM enzymes and sialyation of the BBM with consequent 'gut closure' (Nanthakumar and Henning 1993, Henning 1994, Solomon et al. 2001, Nanthakumar et al. 2003). SI is a marker of intestinal maturation in rats. The activity of SI and SI mRNA is significantly enhanced after DEX treatment in rat pups. GC also induce a decrease in cell turnover (Nanthakumar and Henning 1993). After GC exposure, the immature cells on the intestinal villi were replaced with more mature ones from the crypts (Henning et al. 1994). The same inhibition of crypt-villous kinetics was observed in adult rats exposed to GC. GC stimulates the enterocyte digestive function, but this is concomitant with a reduction of the enterocyte population (Batt and Scott 1982).

Treatment with DEX may be associated with adverse effects. Prenatal and postnatal DEX treatment induces growth retardation in rodents (Okajima et al. 2001, Rooman et al. 1999). DEX administration to adult rats for only 7 days reduces the weight, DNA and protein content of the duodenal, jejunal and ileal mucosa (Park et al.

1994). DEX treatment in adult rats increases bacterial adherence to the intestinal mucosa (Spitz et al. 1994). Interestingly, experiments with rat models of necrotizing enterocolitis (NEC) showed a decreased incidence of this disease if immature rats were previously treated with GC. The permeability of the intestinal mucosa was assessed using serum levels of a fed antigen, ovalbumin (Israel et al. 1990). In small clinical trials with infants, prenatal or postnatal GC administration decreased the incidence of NEC (Halac et al. 1990, Caplan et al. 1993, Vasan et al. 1994). This beneficial effect of DEX has been explained in experiments with rat models by the enhanced maturation and consequent improved barrier function of the intestine.

In experiments with rats treated with DEX, the protein catabolism in the gastrointestinal tract is greater than in skeletal muscle. The DEX-associated catabolism is greater in the ileum than in other regions of the bowel (Burrin et al. 1999). DEX makes the premature ileum vulnerable to perforation, by expanding the volume of the mucosa and thinning of the muscularis externa, with focal degeneration (Gordon et al. 2001). The growth inhibiting effects of DEX in the small intestinal mucosa may be mediated by the redistribution of the IGF-I. After DEX treatment, the amount of IGF-I decreases in the small bowel wall, but increases simultaneous in the villous mucosa. This could explain the intestinal smooth muscle thinning after DEX administration (Gordon et al. 2001).

DEX treatment was associated with somatic growth retardation (Gordon et al. 2001). DEX administrated to preterm infant prevented chronic lung diseases, but caused neuro-developmental problems such as neuromotor dysfunction and severe neurologic defect and/or intellectual defect (Yeh et al. 1998). DEX down-regulates the hepatic

IGF-I endocrine axis, and consequently induces catabolism in the whole body (Kritsch et al. 2002). Exogenous IGF-I increases serum IGF-I concentrations and attenuates DEX-induced catabolism, but does not reduce hepatic levels of IGF-I and IGF-I mRNA. In experiments with parenterally fed rats concomitantly treated with DEX, it was found that IGF-I treatment reduced the whole body catabolism (Lo et al. 1996).

Cellular pathways activated by Glucocorticosteroids

The smooth muscle thinning and the DEX-induced myopathy are due to the blocking activation of DEX on PI-3K, and consequently the inhibition of the anti-apoptotic role of IGF-I (Kritsch et al. 2002). DEX induces the over-expression of the subunit p85α, which is a component of PI-3K. The p85 subunit competes with the PI-3K heterodimer for binding at the IGF-I receptor, and thereby inhibits PI-3K activation. This negative regulation of PI-3K activity decreases the phosphorylation of protein kinase B (PKB), also known as PKB/Akt (Singleton et al. 2000, Bernardi et al. 2001). Mammalian genomes contain three genes encoding PKBs (termed α/AKT1, β/AKT2 and γ). The expression of PKB/Akt appears to be tightly connected with the terminal differentiation of various cell types, and a critical pathway for cell survival (Coffer et al. 1998). Generally, DEX inhibits the growth factor signaling pathway and promotes apoptosis, but this response is dependent on the dose and type of tissue. For example, DEX can decrease apoptosis in the cell culture of osteoblasts by blocking TNF-α induced apoptosis (Chae et al. 2000).

GC are potentially involved in the modulation of cytokines, and this regulation is paralleled with the maturation of the intestinal mucosa (Schaeffer et al. 2000). In the intestinal mucosa, cytokines produced by the epithelium, fibroblasts and immune cells affect differentiation and proliferation. Epithelial-mesenchymal cell interaction plays an important role in differentiation during fetal or adult life (Fritsch et al. 1999, Kedinger et al. 1998). In normal mucosal maturation, SI mRNA increases, and this is paralleled by a rise in TNF-α and a decrease in the exposure of TGF-β1, TGF-β2 and IL-1β. Precocious induction of SI mRNA by hydrocortisone in 11-day-old rats presents the same changes in the levels of cytokines, a decreased expression of TGF-β isoforms and decreased IL-1β. DEX decreases IL-1β but not TGF-β isoforms, *in vivo* as well as *in vivo* (Schaeffer et al. 2000). Recent data have shown that TNF-α increases the biosynthesis of SI in the human epithelial cell line Caco2. However, TNF-α injected *in vivo* before weaning has no effect on SI activity. Also, the expression of TNF-α in the proximal jejunum was not modified by GC *in vivo*. Thus, this cytokine does not seem to be implicated in the onset of SI expression but rather in its subsequent increase. TNF-α may be implicated in the changes in crypt cell proliferation occurring at weaning, because a stimulatory effect on IEC-6 cell growth has been observed (Schaeffer et al. 2000).

Potential applications of Glucocorticosteroids

GC play an important role in the ontogeny of the intestine (see section Glucocorticoid effects on intestinal ontogeny page 107). The normal development and maturation events of the gut take place in the presence of GC. GC have been used clinically to treat immaturity. GC are important in postnatal pulmonary development and surfactant deficiency in low birth or premature infants can be prevented by antenatal or treated with postnatal GC treatment (Bolt et al. 2001). In rat brain, GC promote the transition between the proliferative and differentiating stage, and this permits neuronal and glial maturation events. The action in fetal brain is also mediated via GR and MR receptors, and the repression of 11βHSD2 in late gestation allows GC to act in the brain (Diaz et al. 1998). This benefit is potentially offset by CNS complications such as neuromotor dysfunction (Yeh et al. 1998). Prenatal or postnatal administration of GC decreases the incidence of NEC, probably by the maturation and consequent improved barrier function of the intestine (Halac et al. 1990, Caplan et al. 1993, Vasan et al. 1994). It is known that in experimental work DEX was the favored GC because it doesn't bind to CBG (Solomon et al. 2001). However, as noted previously treatments with DEX could be associated with adverse effects. DEX treatment has an anti-proliferative effect (Croxtall et al. 2002), potentiates apoptosis (Singleton et al. 2000, Bernardi et al. 2001), and causes focal small bowel perforation in low birth infants (Gordon et al. 2001).

1.9 POTENTIAL INTERACTION BETWEEN GLUCOCORTICOSTEROIDS AND GLUCAGON-LIKE PEPTIDE 2

As a generally concept, GC and GLP-2 enhance the intestinal absorption of nutrients. GC amplifies the uptake of hexoses and lipids (Scott et al. 1981, Batt et al. 1982, Keelan et al. 1996, Thiesen 2002). GLP-2 also increases the uptake of hexoses (Cheeseman et al. 1996, 1997, 1998). GC administration in immature intestine induces a precocious functional maturation of the BBM enzymes (Sarantos et al. 1994). GLP-2 administration also enhances the functional activities of the BBM (Brubaker et al. 1997). It is possible that giving both hormones together would lead to an additive or synergistic enhancement of intestinal nutrient absorption, with the proliferative effect of GLP-2 countering the anti- proliferative effect of GC.

GC are known as a major factor in ontogeny, especially in the maturation process (Sarantos et al. 1994). It could be expected that a precocious maturation of the cells by GC during preweaning period will enhance the expression and the activity of GLP-2R.

The role played by GLP-2 in the ontogeny of the intestine is not clear, but the presence of GLP-2R during fetal period suggests a possible function in the development and/or maturation of the gut (Lovshin et al. 2000 and Burrin et al. 2000). Again it may be speculated that GC plus GLP-2 would accelerate intestinal development.

There are other possible pathways by which GC might interact with GLP-2R. GC are nuclear hormones that act mainly through a genomic effect. GC also have rapid non-genomic effects, ‘permissive’ effects which enable other hormones to act on tissues. For example, GC facilitate the actions of catecholamines, thyroid hormones, growth hormone and ACTH (Baxter et al. 1976). GC mediates a permissive action, mostly for hormones acting on ‘G-coupled receptors’, and increase adenyl cyclase activity (Baxter et al. 1976, Haynes et al. 1985, Meier 1997). GC and other nuclear receptors can modulate the transcriptional activity of ligand-activated receptors (Meier 1997). The examples of hyper- and hypothyroidism, Cushing’s syndrome, adrenal insufficiency, and the alterations in G protein signaling which accompanies puberty and menopause provide examples that the basic operation of G protein-linked signaling responds to changes in agents that alter transcription of the gene that encode the receptors, G proteins, and effectors that create their pathways. This cross-talk among different signaling systems was obvious in experiments testing the transcriptional regulation of ‘G-coupled receptors’ by nuclear receptors. Experimental studies of GC action on β-adrenergic receptor mRNA levels provided the first data. For example, DEX increases threefold the β-adrenergic receptor mRNA within two hours (Morris and Malbon 1999).

GLP-2R is a classic GPCR, which belongs to the GLP-1/glucagon/GIP receptor family also known as the GPCR family B of receptors (Munroe et al. 1999). This receptor acts as the other G receptors. The most common effectors of G protein include a) adenyl cyclase; b) cyclic guanosine monophosphate (GMP) phophodiesterases; c) phospolipase C enzymes; d) PI-3K; e) PLA_2; f) ion channels; g) tyrosine kinases and MAPK (Morris and Malbon 1999). GLP-2R increases the level of cAMP in cells without an increase in the intracellular calcium. Activation of this receptor causes an anti-apoptotic signal in culture cells (Brubaker and Drucker 2002).

It is likely that GLP-2R is up-regulated by steroid hormones, since GC enhanced the mRNA level of G coupled receptors. In some experiments, GC increases adenyl cyclase signaling in the absence of changes in G protein subunits, suggesting up-regulation of the expression of the cAMP itself (Meier 1997). A permanent stimulation of G proteins usually causes attenuation of the responses, called ‘desensitization’. Mice treated with GLP-2 injection daily for 12 weeks continued to exhibit enhanced intestinal mass (Tsai et al. 1997). Although GLP-2R desensitization has not yet been examined in this experiment, this possibility cannot be excluded because this phenomenon could be observed for glucagon, GLP-1 and GIP receptors. GC could extend the opposite stimulus and up-regulate a G receptor which was attenuated by its agonist (Hadcock et al. 1989).

GLP-2 could also block some of the effects of GC, such as the decrease in proliferation and the induction of apoptosis. The concomitant administration of growth hormone with DEX might decrease the negative effects of DEX. The combination might preserve the precocious maturation induced by GC treatment without some of the adverse effects of GC. Furthermore, it is possible that this combination of GLP-2 + DEX might have an additive or synergistic effect on the ontogeny of the intestine. If this were the case, then this concomitant treatment of GLP-2 + DEX could be potentially used in the therapy of immature infants with compromised intestinal function.

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CHAPTER 2

HYPOTHESES

HYPOTHESES

1. Glucagon-like peptide 2 plus dexamethasone will enhance *in vitro* lipid uptake more than either agent alone in the immature intestine. This effect is expected when administrated directly to suckling rats, or in offspring of dams exposed to this treatment during lactation or pregnancy plus lactation.

2. Addition of glucagon-like peptide 2 to dexamethasone will protect the small intestine of suckling and weanling (7 weeks old) rats from dexamethasone-induced catabolic effects such as a decrease of body weight and intestinal atrophy.

3. The effects of GLP-2, DEX, or GLP-2 + DEX on lipid uptake of the intestine will persist in later life

CHAPTER 3

TREATMENT OF SUCKLING RATS WITH GLP-2 PLUS DEXAMETHASONE INCREASES THE ILEAL UPTAKE OF FATTY ACIDS IN LATER LIFE

3.1 INTRODUCTION

Genetic programming is the main contributor to the development and maturation of the intestine (Henning et al. 1987, 1994, Clatworthy and Subramanian 2001, Nanthakumar et al. 2003). Various stimuli influence intestinal ontogeny such as hormones, the enteric nervous system, as well as the mesenchyme, and luminal factors which include the luminal bacterial flora and the animals' diet (Pacha et al. 2000, Jensen et al. 2001, Nanthakumar et al. 2003). The digestion and absorption of nutrients demonstrates age-dependent changes during the development of the gut due to alterations in BBM permeability and the BBM nutrient transporters or the enzymes (Henning et al. 1994, Sanderson and Walkers 2000, Buddington and Malo 2003). A diet with a high content in lipids such as milk is well digested during the suckling period in rodents, in spite of a low secretion of lipase and colipase by the newborn pancreas and lack of gastric lipases (Duan 2000). The impaired pancreatic and gastric lipolytic process is compensated for by the lingual and milk lipases (Henning 1987). Lipid absorption is a complex process involving the passive diffusion and protein-mediated transport of lipids across the BBM, their intracellular metabolism, as well as the subsequent transfer of the lipids into the lymphatics or into the portal circulation (Shultess et al. 2000, Besnard et al. 2000). During suckling, the intestine is capable of an increased uptake of lipids as compared with the weanling period (Frost et al. 1983, Flores et al. 1989). This higher uptake results possibly from an increased fluidity of the BBM (Schwarz et al. 1985, Hubner et al. 1988) and/or a greater metabolism of fat in suckling as compared with weanling or adult rats (Frost et al. 1983, Flores et al. 1989). The age-dependent decrease of the BBM fluidity results from alterations in lipid composition of BBM. The suckling intestine contains greater amounts of cholesterol and phospholipids per milligram of protein as compared to the mature intestine (Schwarz et al. 1985, Hubner et al. 1988). The decline in lipid uptake between the suckling and weaning period is associated with a change in the diet from a high uptake of fat in milk, to high uptake of carbohydrates in rat chow.

In adult rats, GLP-2 enhances the intestinal BBM and BLM uptake of sugars (Cheeseman et al. 1996, 1997, 1998) as well as triolein (Brubaker et al. 1997). Also, balance studies in human patients with the short bowel syndrome have shown that GLP-2 increases lipid uptake (Jeppesen et al. 2001). It is not known if this peptide influences the uptake of lipids in suckling animals. GC such as DEX increase the uptake of sugars and lipids in adult rats (Thiesen et al. 2002, 2003), and accelerate the development of the transport and digestive functions of the young intestine (Henning et al. 1994, Pacha et al. 2000). It is not known if the combination GLP-2 + DEX enhances the intestinal uptake of lipids in suckling rats, or if such an effect persists into the post-weaning period.

Accordingly, this study was undertaken to determine 1) if GLP-2 and DEX when administered separately or together to suckling rats enhance the intestinal *in vitro* uptake of lipids; 2) if these changes in the uptake of lipids are due to variations in the intestinal mass or morphology, or to changes in the abundance of selected intracellular lipid binding proteins in the enterocytes; and 3) if the effects of GLP-2 and DEX on the intestine persist into the post-weaning period.

3.2 MATERIALS AND METHODS

3.2.1. Animals

The principles for the care and use of laboratory animals, approved by the Canadian Council on Animal Care and by the Council of the American Physiological Society, were observed in the conduct of this study. All experiments were approved by the Animal Ethics Board, University of Alberta. Eight nursing Sprague Dawley rats with 64 suckling offspring (8 offspring/dam) were obtained from Bio Science Animal Services, University of Alberta.

The suckling rats were randomized into four groups (n=16), which received treatment with GLP-2, DEX, GLP-2 plus DEX, or Placebo. All animals received treatment for 10 days starting with the 11th day from time of their delivery. Rat GLP-2 was purchased from American Peptide Sunnyvale CA and DEX from Sigma-Aldrich Canada. GLP-2 was administrated in a dose of 0.1 μg/g body weight/day subcutaneously [sc] twice per day at 7am and 7 pm. DEX was administrated in a dose of 0.128 μg/g body weight/day sc once per day at 7 pm. The regimen used for GLP-2 + DEX group was GLP-2 0.1 μg/g body weight/day sc twice per day at 7am and 7 pm, plus DEX 0.128 μg/g body weight/day sc once per day at 7 pm. The placebo group received 0.9% saline in a volume equal to the volume of GLP-2 administrated daily per rat (depending on the weight of the rat, the volume ranged from 0.08 ml in the first day to 0.18ml in the 10th day), sc, twice per day at 7 am and 7 pm.

There were 16 animals in each group. Following treatment, 32 sucklings were sacrificed for uptake studies at 21 days of age, and 32 post-weaning animals ("weanlings") were sacrificed for the uptake studies at 7 weeks of age [Figure 7].

The animals were housed at a temperature of 21°C, and in each day they were exposed to 12 hours of light and 12 hours of darkness. During the suckling period, the offspring received only the dam's milk because the food was too far to be reached by the small rats. The weanlings were housed in pairs. Their water and food were supplied *ad libitum*. The dams and the weanlings were fed standard rat chow, PMI # 5001 (Nutrition International LLC, Brentwood, MO, USA). The diets were nutritionally adequate, providing for all known essential nutrient requirements. Body weights were recorded at the time of weanling and then weekly for the next four weeks.

3.2.2. Uptake Studies

Probe and marker compounds

The [^{14}C]-labeled probes included cholesterol (0.05 mM) and six fatty acids (0.1 mM): lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The labeled and unlabeled probes were supplied by Amersham Biosciences Inc. (Baie d'Urfe, PQ). The lipid probes were prepared by solubilizing them in 10 mM taurodeoxycholic acid (Sigma Co., St Louis, MO) in Krebs-bicarbonate buffer, with the exception of 12:0 which was solubilized only in Krebs-bicarbonate buffer. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume (Westergaard and Dietschy 1974).

Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl® (sodium pentobarbital, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed and rinsed with 150 ml cold saline. The intestine was divided into two parts: the proximal half of the intestine beginning at the ligament of Treitz was termed the "jejunum", and the distal half was termed the "ileum". A 2 cm piece of each segment of jejunum and ileum was gently scraped with a glass slide. The mucosal scrapings and the remaining wall of the intestine were dried overnight in an oven at 55°C. The percentage of the intestinal wall comprised of mucosa was calculated. The remaining intestine was everted, and cut into small rings of approximately 2-4 mm each. These intestinal rings were immersed in pre-incubation beakers containing Krebs's buffer (pH 7.2) at 37°C, bubbled with oxygen plus bicarbonate (O_2-CO_2, 95:5 by volume), and were allowed to equilibrate for 5 minutes (Perin et al. 1997). Uptake was initiated by the timed transfer of the tissue rings from the pre-incubation buffer to a 5 ml plastic vial containing [^{3}H]-inulin and [^{14}C]-labelled lipids in Kreb's buffer bubbled with oxygen plus bicarbonate that had been equilibrated to 37°C in a shaking water bath. The intestinal rings were incubated in the lipid substrates for 5 minutes.

Determination of uptake rates

The rate of uptake of lipid was terminated by pouring the vial contents onto filters on an Amicon vacuum filtration manifold that was maintained under suction, followed by washing the intestinal rings three times with ice-cold saline. The tissue rings were placed on a glass slide, and were dried overnight in an oven at a constant temperature of 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M NaOH, scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes (Westergaard and Dietschy 1974). The rates of lipid uptake were determined as nmol of substrate absorbed per 100 mg dry weight of the whole intestinal wall per minute (Jd, nmol •100 mg tissue $^{-1}$• min $^{-1}$), or as nmol of substrate absorbed per 100 mg dry weight of the mucosa per minute (Jm, nmol•100 mg mucosal tissue $^{-1}$• min $^{-1}$).

3.2.3. Morphological analysis

In order to determine the morphological characteristics of the intestine, a vertical section was prepared from the jejunum and from the ileum. Hematoxylin and eosin-stained slides were prepared from paraffin blocks. Crypt depth, villous height, villous width, villous width at half height, and cell density were measured using the program MetaMorph 5.05r (Universal Imaging Corporation, Downingtown, PA, USA). The group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of four animals in each group.

3.2.4. Immunohistochemistry

Jejunal and ileal tissues were embedded in paraffin, and 4-5 micron sections were mounted on glass slides. The sections were heated and placed immediately in xylene (2 x for 5 min each), followed by absolute ethanol (2 x for 2 min each), and were then rinsed with tap water. The slides were incubated in a hydrogen peroxide/methanol solution, and rinsed with tap water. Then they were rehydrated, and the tissue was encircled on the slides with a hydrophobic slide marker (PAP pen, BioGenex, California). The slides were incubated for 15 min in blocking reagent (20% normal goat serum) followed by primary antibody to I-FABP or to L-FABP for 30 min. Both antibodies were a generous gift from Dr. LB Agellon, University of Alberta. The slides were incubated in LINK® and LABEL®, and with DAB® solution (BioGenex, San Ramon, California). The slides were then washed, stained in hematoxylin, dehydrated in absolute ethanol, and cleared in xylene. The slides were photographed using AxioCam MRc (Zeiss), and the area labeled with antibody was determined using MetaMorph 5.05r. The results were expressed as a ratio of the area that was antibody-positive versus the total area. Statistical analyses were based on a minimum of four villi per animal, and four animals per group.

3.2.5. Statistical analyses

The results obtained were expressed as mean ± standard error of the mean. The statistical significance of the differences between the four groups was determined by analysis of variance (ANOVA) for values of $p \leq 0.05$. The significant differences between sucklings versus weanlings were determined using a Student's t-test. Statistical significance was accepted for values of $p \leq 0.05$.

3.3 RESULTS

3.3.1. Body and Intestinal Weights, and Villous Morphology

Injecting GLP-2, DEX or GLP-2 + DEX into the suckling animals for 10 days had no effect on the animal's body weight (Table 5). In contrast, the mean body weight of the weanlings injected six weeks previously with DEX was approximately 25% lower than in the control animals. When GLP-2 was given with DEX, the decrease in body weight in the weanlings given DEX was not observed.

Giving GLP-2, DEX, or GLP-2 + DEX to the suckling rats had no influence on the jejunal or ileal weight, weight of the mucosa, or percentage of the intestinal wall comprised of mucosa (data not shown). In contrast, in weanlings the total ileal weight was increased from 7.6 ± 0.8 mg/cm in controls to 10.1 ± 0.8 mg/cm ($p < 0.05$) in those given GLP-2 (Table 6). Neither DEX nor GLP-2 + DEX influenced the intestinal characteristics in weanlings.

GLP-2 increased the crypt depth in the jejunum of sucklings, whereas DEX as well as GLP-2 + DEX increased the jejunal villous height, width at the base, and crypt depth (Table 7). In the ileum of sucklings, GLP-2 decreased the distance between villi, DEX by itself had no effect, whereas GLP-2 + DEX increased all the morphological parameters of the intestine (Table 8).

In the jejunum of weanlings, GLP-2, DEX, and GLP-2 + DEX increased the distance between cells, indicating an increase in enterocyte size; GLP-2 + DEX also increased the villous height (Table 9). In the ileum of weanlings, GLP-2 by itself had no effect, DEX increased the villous height and cell size, whereas GLP-2 + DEX decreased the villous width, crypt depth, distance between villi, and distance between 10 cells (Table 10).

3.3.2. Lipid uptake

Because of the influence of GLP-2 and DEX on the morphology and characteristics of the intestine (Tables 6-10), the lipid uptake was expressed on the basis of the weight of the mucosa (Jm, mmol/100mg mucosa $^{-1}$ /minute $^{-1}$) as well as on the basis of the weight of the intestinal wall (Jd, mmol/100mg $^{-1}$/min $^{-1}$).

In sucklings, GLP-2 had no effect on the jejunal or ileal uptake (Jd or Jm) of lipids (Tables 6 and 7). DEX increased the uptake (Jd and/or Jm) of 18:2 and 18:3 in the jejunum, as well as the uptake (Jd) of 16:0 in the ileum. GLP-2 + DEX increased the jejunal uptake (Jd) of 18:0 and 18:2, but had no effect on ileal uptake.

In weanlings, four weeks after the treatment with GLP-2, there was no difference in the jejunal or ileal uptake of lipids (Jd or Jm). DEX increased the ileal uptake (Jd and /or Jm) of 16:0 and 18:0. GLP-2 + DEX increased the ileal uptake (Jd and /or Jm) of 12:0, 18:0, 18:1, 18:2 and 18:3(Tables 13 and 14).

The jejunal and ileal uptake (Jd and /or Jm) of 12:0, 16:0, 18:0, 18:2, and cholesterol was lower in weanlings than in sucklings (Tables 15-18). Giving GLP-2, DEX or GLP-2 +DEX directly to the suckling rats did not affect this age-dependent decline in lipid uptake.

3.3.3. Immunohistochemistry

GLP-2+DEX did not change the abundance of I-FABP or L-FABP in the ileum of the weanlings (Table 19).

3.4 DISCUSSION

GLP-2 in a dose of 5μg/day (approximately 0.16 μg/g body weight/day, slightly greater than our dose of 0.1 μg/g body weight/day) and given for 10 days enhances the uptake of C^{14}triolein in adult mice (Brubaker et al. 1997), and improves fat absorption measured by balance studies in humans (GLP-2 in a dose of approximately 0.01 μg/g body weight/day) (Jeppesen et al. 2001). Also, the absorption of other nutrients such as leucine (Brubaker et al. 1997) and sugars (Cheeseman et al. 1996, 1997, 1998) is enhanced in adult rats after the administration of GLP-2. When GLP-2 treatment was given for 10 days to the suckling rats, there was no effect on lipid uptake (Tables 11 and 12). GLP-2 had no effect on body weight in the suckling or weanling rats (Table 5), although in previous studies GLP-2 enhanced the body weight of adults (Scott et al. 1998, Jeppesen et al. 2001). Also, GLP-2 has a trophic action on the adult intestine (Drucker 1996, 2003). In sucklings, the total weight of the ileum was increased as well as the crypt depth (Tables 6 and 7). Furthermore, when GLP-2 is given with DEX, there was a marked enhancement in the ileal morphology in the suckling rats (Table 8). This suggests that GLP-2 given to suckling rats has a modest trophic effect, which may be facilitated by the co-administration of DEX.

This very modest effect of GLP-2 on intestinal morphology when given alone was surprising, since GLP-2Rs are present in the intestine of young rats, and in fact their level is higher than in adults (Lovshin et al. 2000, Burrin et al. 2000). Of interest, Petersen and coworkers (2001) showed a lack of any trophic effect for GLP-2 in the intestine of pigs before birth. Our data suggest that the GLP-2Rs in the young animals are capable of responding to this dose of GLP-2 with only very modest changes in the characteristics of the intestine, but with no alteration in lipid uptake. Clearly, it is unlikely that GLP-2 would be useful to accelerate intestinal morphological development or to enhance lipid absorption in young animals.

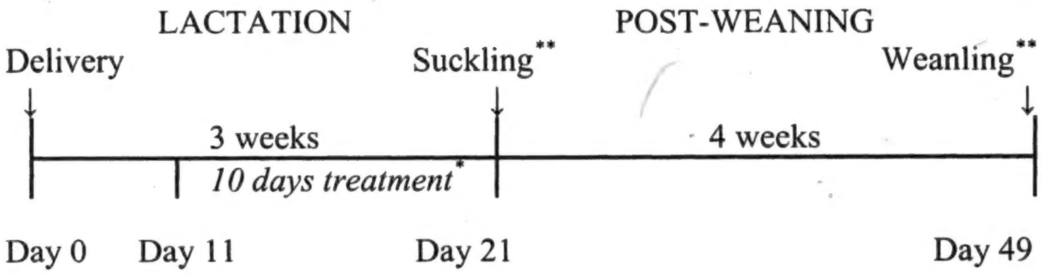
GC such as DEX are recognized as being capable of accelerating the ontogeny of the intestine, including enhancing the absorption of nutrients such as sugars and amino acids (James et al. 1987, Buchmiller et al. 1994, Ianolli et al. 1998). GC also enhance the intestinal uptake of lipids in adult rats (Thiesen et al. 2002, 2003). DEX increased the uptake of several lipids in suckling rats (Tables 11 and 12), and in the jejunum this was associated with an increase in villous height, villous width, and crypt depth (Table 7). Thus, DEX is capable of stimulating intestinal lipid uptake in sucklings, similar to what has been reported previously in adults.

A major finding in this study was the stimulating effect of GLP-2 + DEX on lipid uptake into the ileum, not at the time of injection, but rather four weeks later in weanlings (Tables 13 and 14). This was not obviously due to any change in body weight (Table 5), intestinal characteristics (Table 6), villous morphology (Table 10), or the abundance of the enterocyte lipid binding proteins I- or L-FABP (Table 19). Others have also observed that changes in I-FABP abundance do not necessary follow alterations in lipid uptake (Agellon et al. 2002, Vassileva et al. 2000).

The late effect of early nutrition has been described previously and the concept of "critical period programming" is documented (Karasov et al. 1985, Pacha et al. 2000). For example, changes in the dietary content of carbohydrates, lipids and proteins during the suckling or weanling period influence the morphology and/or the nutrient uptake in the intestine (Karasov et al. 1985, Thomson et al. 1987, 1989). Moreover, the maternal diet during pregnancy and nursing influences the morphological development and the absorption of nutrients in the offspring (Jarocka-Cyrta et al. 1998, Perin et al. 1999).

Thus, while GLP-2 does not appear to be stimulating to the suckling intestine, it does have an effect on the morphology and function of the post-weaning intestine when combined with DEX. These studies did not establish the mechanism(s) of this late effect of GLP-2 + DEX. The persistent enhanced uptake of lipids (Tables 13 and 14) and trophic changes in the crypt-villous morphology (Tables 9 and 10) seen in the weanlings exposed one month previously to GLP-2 + DEX raises the possibility that these alterations may continue into later life, and could thereby potentially contribute to unwanted abnormalities in lipid metabolism. It would be important for future studies to clarify this issue, and especially to determine if there is continued hyper-absorption of lipids, and if this has any adverse influence on the animal's later body weight, or the development of hyperlipidemia.

Figure 7. Experiment 1 design


LACTATION
POST-WEANING
Delivery
Suckling**
Weanling**
3 weeks
4 weeks
10 days treatment*
Day 0
Day 11
Day 21
Day 49

* Treatment with GLP-2, DEX, GLP-2 + DEX, and Placebo was administrated each day during lactation.

** Uptake studies were performed at day 21 ("suckling") and day 49 ("weanling").

Table 5. Body weights of suckling and weanling rats injected for 10 days during lactation with GLP-2, DEX, GLP-2 + DEX, or Placebo

	CONTROL	**GLP-2**	**DEX**	**GLP-2+DEX**
SUCKLING	45.5 ± 0.8	46.3 ± 1.1	42.1 ± 1.3	44.2 ±1.3
WEANLING	213.2 ± 13.4a	204.5 ± 6.5a	162.7 ± 2.9b	200.6 ± 12.0a

Values are expressed mean ± sem. There were 8 rats in each group
a, b : values with different letters are significantly different $p < 0.05$ by ANOVA
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to suckling animals in the last for 10 days of the lactation. The sucklings were sacrificed on day 21 and the weanlings were sacrificed 28 days after the weaning. There were 8 rats per treatment group

Table 6. Intestinal characteristics of weanling rats injected 10 days during lactation with GLP-2, DEX, GLP-2 + DEX, or Placebo

		CONTROL	GLP-2	DEX	GLP-2+DEX
JEJUNUM	Mucosa (mg/cm)	8.0 ± 0.8	8.1 ± 1.0	5.7 ± 0.5	7.8 ± 1.1
	Submucosa (mg/cm)	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.0 ± 0.2
	Total Weight (mg/cm)	8.9 ± 0.9	9.2 ± 0.9	6.8 ± 0.5	8.8 ± 1.0
	% Mucosa	88.8 ± 2.0	86.0 ± 3.0	84.1 ± 2.3	86.1 ± 3.3
ILEUM	Mucosa (mg/cm)	6.7 ± 0.8	8.2 ± 1.0	5.0 ± 0.5	5.7 ± 0.8
	Submucosa (mg/cm)	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2
	Total Weight (mg/cm)	7.6 ± 0.8 a	10.1 ± 0.8 b	6.1 ± 0.5 a	7.0 ± 0.7 a
	% Mucosa	87.5 ± 2.8	88.2 ± 1.6	81.9 ± 2.4	80.0 ± 3.0

Values are expressed mean ± sem.
a, b : values with different letters are significantly different $p < 0.05$ by ANOVA. The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given for 10 days during lactation. The weanling animals were sacrificed on day 49. There were 8 weanling rats per treatment group.

Table 7. The effect of treatment of suckling rats with GLP-2, DEX and GLP-2 + DEX on jejunal morphology

Jejunum (µm)	**Control**			**GLP2**			**DEX**			**GLP2+DEX**		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	352.7	±	12.0**ab**	300.8	±	8.7**a**	486.4	±	5.6**c**	406.2	±	39.1**b**
Villous Width (base)	75.0	±	5.1**a**	72.9	±	2.9**a**	105.4	±	1.0**b**	105.0	±	4.8**b**
Villous Width (half height)	68.8	±	5.0	75.7	±	0.1	61.1	±	2.6	76.4	±	9.7
Crypt Depth	53.9	±	5.7**a**	66.1	±	3.7**b**	70.5	±	0.5**b**	74.0	±	1.6**b**
Distance /5 villi	436.3	±	24.4	424.4	±	25.0	509.8	±	10.7	533.2	±	50.1
Distance /5 cells	39.0	±	6.0	25.7	±	2.3	31.3	±	4.2	29.1	±	1.2

Values are mean ± sem

a, b: values with different letters are significantly different $p<0.05$ by t-test.

The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The suckling rats were sacrificed on day 21. In each group there were 8 suckling rats.

Table 8. The effect of treatment of suckling rats with GLP-2, DEX and GLP-2 + DEX on ileal morphology

Ileum (µm)	**Control**			**GLP2**			**DEX**			**GLP2+DEX**		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	236.9	±	33.5**a**	175.2	±	18.1**a**	261.3	±	59.5**a**	443.8	±	14.1**b**
Villous Width (base)	72.5	±	10.4**a**	62.4	±	2.1**a**	86.7	±	7.8**a**	160.7	±	11.7**b**
Villous Width (half height)	72.0	±	6.8**a**	62.5	±	2.5**a**	62.2	±	5.6**a**	126.6	±	12.0**b**
Crypt Depth	49.8	±	4.8**a**	52.3	±	4.8**a**	69.7	±	5.8**a**	124.2	±	13.6**b**
Distance /5 villi	455.8	±	34.4**b**	348.2	±	15.7**a**	424.9	±	29.3**ab**	958.3	±	27.6**c**
Distance /5 cells	27.4	±	5.2**a**	24.9	±	1.0**a**	23.9	±	0.8**a**	45.0	±	1.7**b**

Values are mean ± sem
a, b: values with different letters are significantly different $p<0.05$ by t-test.
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The suckling rats were sacrificed on day 21. In each group there were 8 suckling rats.

Table 9. The effect of treatment of suckling rats with GLP-2, DEX and GLP-2 + DEX on jejunal morphology of the weanling rats

Jejunum (µm)	**Control**			**GLP2**			**DEX**			**GLP2+DEX**		
Weanling	**Mean**		**Sem**	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	386.4	±	12.8**a**	383.4	±	18.0**a**	370.0	±	28.1**a**	462.7	±	19.9**c**
Villous Width (base)	126.5	±	7.7	115.3	±	10.0	125.1	±	3.1	113.2	±	4.4
Villous Width (half height)	105.3	±	1.7	109.9	±	6.3	86.5	±	7.8	85.4	±	7.3
Crypt Depth	91.6	±	3.1	91.1	±	3.6	91.5	±	6.9	93.2	±	6.1
Distance /5 villi	695.4	±	65.0	633.4	±	74.8	556.0	±	22.3	527.6	±	18.7
Distance /5 cells	24.3	±	1.3**a**	33.2	±	1.7**b**	33.4	±	0.7**b**	39.0	±	1.8**c**

Values are expressed mean ± sem.

a, b: values with different letters are significantly different $p<0.05$ by t-test.

The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The weanlings were sacrificed 28 days after weanling. In each group were 8 rats.

Table 10. The effect of treatment of suckling rats with GLP-2, DEX and GLP-2 + DEX on ileal morphology of the weanling rats

Ileum (μm)	**Control**			**GLP2**			**DEX**			**GLP2+DEX**		
Weanling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	203.2	±	10.7**a**	149.3	±	37.9**a**	281.4	±	13.5**b**	123.8	±	13.6**a**
Villous Width (base)	104.0	±	5.1**a**	92.3	±	24.7**a**	113.0	±	7.5**a**	45.6	±	3.8**b**
Villous Width (half height)	86.9	±	2.5**a**	76.4	±	16.7**a**	80.5	±	5.3**a**	43.5	±	3.2**b**
Crypt Depth	89.6	±	7.3**a**	68.2	±	7.1**a**	89.6	±	11.7**a**	38.3	±	1.8**b**
Distance /5 villi	593.9	±	27.6**a**	460.9	±	98.3**a**	593.4	±	24.5**a**	325.8	±	54.1**b**
Distance /5 cells	24.1	±	1.3**b**	17.9	±	2.2**b**	39.3	±	1.3**c**	13.4	±	0.6**a**

Values are mean ± sem
a, b: values with different letters are significantly different $p<0.05$ by ANOVA.
The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The weanlings were sacrificed 28 days after weanling. In each group were 8 rats.

Table 11. Effect of treatment of suckling rats on jejunal and ileal uptake (Jd) of lipids in suckling rats

Jejunum	FA	Control mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	25.1±2.1	26.7±5.0	36.1±3.8	28.4±3.8
	16:0	1.5±0.1	1.5±0.2	2.3±0.2	2.2±0.3
	18:0	1.4±0.2 b	1.5±0.2 b	2.2±0.3 ab	2.6±0.3 a
	18:1	1.6±0.3	1.2±0.2	1.9±0.3	2.2±0.2
	18:2	1.0±0.1 b	1.5±0.2ab	1.8±0.3 a	2.2±0.3 a
	18:3	1.3±0.2	1.8±0.3	1.9±0.2	1.9±0.3
	Chol	1.9±0.2	1.2 ±0.3	2.4±0.6	2.1±0.6
Ileum	12:0	30.4 ±6.7	45.6±7.7	55.2±5.8	36.3±3.9
	16:0	2.4±0.3 b	2.1±0.4 b	4.3±0.5 a	2.0±0.3 b
	18:0	2.2±0.4	2.3±0.4	3.3±0.4	2.8±0.3
	18:1	2.3±0.4	2.6±0.4	2.9±0.6	2.6±0.4
	18:2	2.6±0.3	2.0±0.3	3.6±0.5	2.4±0.4
	18:3	1.9±0.3	2.3±0.3	2.3±0.3	2.8±0.4
	Chol	3.3±0.7	2.2±0.8	2.8±0.8	3.5±0.8

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given for 10 days during lactation to the suckling rats. The suckling animals were sacrificed on day 21 and there were 8 suckling rats in each group.

Table 12. Effect of treatment of suckling rats on jejunal and ileal uptake (Jm) of lipids in suckling rats

Jejunum	FA	Control mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	10.0±1.3	14.2±2.6	13.2±2.3	11.9±1.0
	16:0	0.6±0.1	0.8±0.2	0.8±0.1	0.9±0.1
	18:0	0.6±0.1	0.9±0.1	0.8±0.1	1.1±0.2
	18:1	0.7±0.2	0.7±0.1	0.8±0.2	0.9±0.1
	18:2	0.5±0.1	0.3±0.4	0.9±0.2	1.0±0.2
	18:3	0.5±0.1 b	0.9±0.1 ab	1.2±0.3 a	0.7±0.1 ab
	Chol	1.9±0.2 ab	0.7±0.3 b	2.2±0.7 a	0.9±0.3 ab
Ileum	12:0	17.0±2.9	23.7±3.6	30.9±4.1	22.5±3.3
	16:0	1.1±0.2	0.5±0.6	1.7±0.2	0.7±0.1
	18:0	1.1±0.2	1.2±0.2	1.4±0.2	1.6±0.2
	18:1	1.1±0.2	1.3±0.2	1.4±0.3	1.3±0.2
	18:2	0.9±0.1	1.0±0.2	1.1±0.2	1.1±0.1
	18:3	0.8±0.1	1.3±0.2	1.5±0.4	1.2±0.2
	Chol	3.3±0.7	1.9±0.8	4.6±1.5	1.2±0.3

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given for 10 days during lactation to the suckling rats. The suckling animals were sacrificed on day 21 and there were 8 suckling rats in each group.

Table 13. Effect of treatment of suckling rats on jejunal and ileal uptake (Jd) of lipids in weanling rats

Jejunum	FA	Control mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	12.4±1.3	13.2±1.1	12.3±1.2	12.4±0.9
	16:0	2.0±0.2 a	1.8±0.1 a	1.8±0.3 a	1.2±0.1 b
	18:0	1.5±0.2	1.5±0.1	1.4±0.2	1.0±0.1
	18:1	1.7±0.2	1.6±0.1	1.5±0.2	1.5±0.2
	18:2	1.6±0.2	1.0±0.1	1.4±0.2	1.0±0.1
	18:3	1.5±0.1	1.5±0.2	1.4±0.2	1.2±0.2
	Chol	1.5±0.2	1.5 ±0.1	1.5±0.2	1.0±0.1
Ileum	12:0	14.6 ±1.1b	15.7±1.7 b	14.5±2.0 b	21.2±1.4 a
	16:0	1.3±0.1 b	1.6±0.2 ab	2.1±0.3 a	1.4±0.1 b
	18:0	1.3±0.1 b	1.8±0.2 b	3.1±0.4 a	1.3±0.1 b
	18:1	2.1±0.3	1.4±0.2	1.5±0.2	2.0±0.2
	18:2	1.2±0.2 ab	0.8±0.1 b	1.6±0.2 a	1.5±0.1 a
	18:3	1.1±0.2	1.2±0.1	1.4±0.2	1.3±0.1
	Chol	2.4±0.3 a	1.8±0.2 ab	2.4±0.2 a	1.4±0.1 b

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$. Jd by ANOVA: uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given for 10 days during lactation to the suckling rats. The weanling animals were sacrificed on day 49 and there were 8 suckling rats in each group.

Table 14. Effect of treatment of suckling rats on jejunal and ileal uptake (Jm) of lipids in weanling rats

Jejunum	FA	Control mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	8.8±0.8	10.0±1.1	7.4±0.7	9.6±1.0
	16:0	1.2±0.1	1.2±0.1	0.8±0.1	0.9±0.1
	18:0	0.9±0.1	1.0±0.1	0.8±0.1	0.9±0.1
	18:1	1.1±0.1 ab	1.1±0.1 ab	1.0±0.1 b	1.5±0.2 a
	18:2	0.7±0.1 ab	0.6±0.1 b	0.6±0.1 b	1.0±0.2 a
	18:3	0.9±0.1	1.0±0.1	0.7±0.1	1.1±0.2
	Chol	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1
Ileum	12:0	9.8±1.0 b	9.7±1.2 b	8.3±0.8 b	16.5±1.4 a
	16:0	0.8±0.1	1.1±0.1	1.2±0.1	1.1±0.1
	18:0	0.8±0.1 b	1.1±0.1 b	1.4±0.1 a	1.1±0.1 a
	18:1	1.0±0.1 b	0.9±0.1 b	0.8±0.1 b	1.6±0.2 a
	18:2	0.7±0.1 b	0.6±0.1 b	0.9±0.1 b	1.3±0.1 a
	18:3	0.7±0.1b	0.8±0.1 b	0.7±0.1 b	1.2±0.1 a
	Chol	1.4±0.2	1.3±0.1	1.1±0.1	1.1±0.1

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given for 10 days during lactation to the suckling rats. The weanling animals were sacrificed on day 49 and there were 8 suckling rats in each group.

Table 15. Comparison of lipid uptake (Jd) into the jejunum of suckling vs weanling rats

FA	**Control**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	25.1±2.1	12.4±1.3#	26.7±5.0	13.2±1.1#	36.1±3.8	12.3±1.2#	28.4±3.8	12.4±0.9#
16:0	1.5±0.1	2.0±0.2#	1.5±0.2	1.8±0.1	2.3±0.2	1.8±0.3	2.2±0.3	1.2±0.1#
18:0	1.4±0.2	1.5±0.2	1.5±0.2	1.5±0.1	2.2±0.3	1.4±0.2#	2.5±0.3	1.0±0.1#
18:1	1.6±0.3	1.7±0.2	1.2±0.2	1.6±0.1	1.9±0.3	1.5±0.2	2.2±0.2	1.5±0.2#
18:2	1.0±0.1	1.6±0.2#	1.5±0.2	1.1±0.1	1.8±0.3	1.4±0.2	2.2±0.3	1.0±0.1#
18:3	1.3±0.2	1.5±0.1	1.8±0.3	1.51±0.2	1.9±0.2	1.4±0.2	1.9±0.3	1.2±0.2#
Chol	1.9±0.2	1.5±0.1	1.2±0.3	1.5±0.1	2.4±0.6	1.5±0.2	2.1±0.6	1.0±0.1

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The sucklings were sacrificed on day 21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats

Table 16. Comparison of lipid uptake (Jm) into the jejunum of suckling vs weanling rats

FA	**Control**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	10.0±1.32	8.8±0.8	14.2±2.3	10.0±1.1	13.2±2.3	7.4±0.7#	11.9±1.0	9.6±1.0
16:0	0.620.1	1.2±0.1#	0.8±0.2	1.2±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.9±0.1
18:0	0.6±0.1	0.9±0.1	0.9±0.1	1.0±0.1	0.8±0.1	0.8±0.1	1.1±0.2	0.9±0.1
18:1	0.7±0.2	1.1±0.1#	0.7±0.1#	1.1±0.1#	0.8±0.2	0.9±0.1	0.9±0.1	1.5±0.2#
18:2	0.5±0.1	0.7±0.1#	0.3±0.4	0.6±0.1	0.9±0.2	0.6±0.1	1.0±0.2	1.0±0.2
18:3	0.5±0.1	0.9±0.1#	0.9±0.1	1.0±0.1	1.2±0.3	0.7±0.1	0.7±0.1	1.1±0.2
Chol	1.9±0.2	0.9±0.1#	0.7±0.3	0.9±0.1	2.2±0.7	0.9±0.1#	0.9±0.3	0.9±0.1

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The sucklings were sacrificed on day 21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats

Table 17. Comparison of lipid uptake (Jd) into the ileum of suckling vs weanling rats

FA	**Control**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	30.4±6.7	14.6±1.1#	45.6±7.7	15.7±1.7#	55.2±5.8	14.5±2.0#	36.3±3.9	21.2±1.4#
16:0	2.4±0.3	1.3±0.1#	2.1±0.4	1.6±0.2	4.3±0.5	2.1±0.3#	1.9±0.3	1.4±0.1
18:0	2.2±0.4	1.3±0.1#	2.3±0.4	1.8±0.2	3.3±0.4	3.1±0.4	2.8±0.3	1.3±0.1#
18:1	2.3±0.4	2.1±0.3	2.6±0.4	1.4±0.2#	2.9±0.6	1.5±0.2#	2.6±0.4	2.0±0.2
18:2	2.6±0.3	1.2±0.2#	2.0±0.3	0.8±0.1#	3.6±0.5	1.6±0.2#	2.4±0.4	1.5±0.1#
18:3	1.9±0.3	1.1±0.2	2.3±0.3	1.2±0.1#	2.3±0.3	1.4±0.2#	2.8±0.4	1.3±0.1#
Chol	3.3±0.7	2.4±0.3	2.3±0.8	1.8±0.2	2.8±0.8	2.4±0.2	3.5±0.8	1.4±0.1#

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The sucklings were sacrificed on day 21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats

Table 18. Comparison of lipid uptake (Jm) into the ileum of suckling vs weanling rats

FA	**Control**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	17.0±2.9	9.8±1.0#	23.7±3.6	9.7±1.2#	30.9±4.1	8.3±0.8#	22.5±3.3	16.5±1.4
16:0	1.1±0.2	0.8±0.1	0.5±0.7	1.1±0.1	1.7±0.2	1.2±0.1	0.7±0.1	1.1±0.1#
18:0	1.1±0.2	0.8±0.1	1.2±0.2	1.1±0.1	1.4±0.2	1.4±0.1	1.6±0.2	1.1±0.1#
18:1	1.1±0.2	1.0±0.1	1.3±0.2	0.9±0.1	1.4±0.3	0.8±0.1	1.3±0.2	1.6±0.2
18:2	0.9±0.1	0.7±0.1	1.0±0.2	0.6±0.1	1.1±0.2	0.9±0.1	1.1±0.1	1.3±0.1
18:3	0.8±0.1	0.7±0.1	1.3±0.2	0.8±0.1#	1.5±0.4	0.7±0.1#	1.2±0.2	1.2±0.1
Chol	3.3±0.7	1.4±0.2#	2.0±0.8	1.3±0.1	4.6±1.5	1.1±0.1#	1.2±0.3	1.1±0.1

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given to the suckling rats for 10 days during lactation. The sucklings were sacrificed on day 21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 19. The effect of GLP-2 + DEX on the abundance of I-FABP and L-FABP in the ileum of weanlings

Weanling	CONTROL			GLP-2+DEX		
I-FABP	69.5	±	3.0	71.3	±	2.5
L-FABP	62.4	±	3.3	70.3	±	0.9

Values are ratio of area stained with antibody versus total tissue area and are expressed as mean ± sem.

There were no significant differences between groups when tested by t-test

The regimen used for GLP-2 + DEX was GLP-2 0.1 μg / g twice a day plus DEX 0.128 μg / g once a day, given to the suckling rats for 10 days during lactation. The weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

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CHAPTER 4

DEXAMETHASONE PLUS GLP-2 GIVEN TO LACTATING RAT DAMS HAS A LATE EFFECT ON INTESTINAL LIPID UPTAKE IN THE WEANLING OFFSPRING

4.1 INTRODUCTION

Lipid absorption is the result of the sum of the processes of passive diffusion as well as protein-mediated transport (Besnard et al. 2000, Shultess et al. 2000). There is little information about the early development of lipid absorption in the intestine. Rats and mice have lingual lipases but no gastric lipases (Duan 2000). The pancreas of the newborn has a low secretion of lipase and colipase, which is partially compensated for by the lingual as well as milk lipases (Henning 1987). Also, this small capacity for digesting lipids is partially compensated for by a higher intestinal uptake of lipids in sucklings than in adults. This is due in part to an increased fluidity of the BBM (Schwarz et al. 1985, Hubner et al. 1988), which allows for an enhanced passive diffusion of lipids. Moreover, there is a greater metabolism of fat in suckling as compared with adult rats (Frost et al. 1983, Flores et al. 1989). During the ontogeny of the gut, there are age-dependent changes in the absorption of carbohydrates, lipids and amino acids, which prepare the intestine for the diet changes which occur between suckling and weanling. These alterations in absorption are possibly due to changes in the nutrient transporters, digestive enzymes and BBM permeability (Sanderson and Walkers 2000, Henning et al. 1994, Buddington and Malo 2003). The interactions between diverse stimuli and genetic programming contribute to the morphological and functional maturation of the intestine (Henning et al. 1987, 1994, Clatworthy and Subramanian 2001, Nanthakumar et al. 2003, Roffler et al. 2003). These stimuli are induced by hormones, the enteric nervous system, the mucosa and mesenchyme, as well as by luminal factors such as diet and intestinal bacterial flora (Pacha et al. 2000, Jensen et al. 2001, Nanthakumar et al. 2003).

In adult animals, GLP-2 enhances the absorption of sugars (Cheeseman et al. 1996, 1997, 1998), amino acids such as leucine, and lipids such as triolein (Brubaker et al. 1997). It is not known if GLP-2 influences the absorption of lipids in young animals. Also, GC such as DEX increase the intestinal uptake of sugars and lipids in adult rats (Thiesen et al. 2002, 2003), but it is not known if DEX alters lipid uptake in sucklings. Previous studies with rodents have shown that the lipid content of the maternal diet during pregnancy or lactation influences the development and the absorption of nutrients in the offspring (Jarocka-Cyrta et al. 1998, Perin et al. 1999). Some lactating mothers might be given GLP-2 for treatment of the short bowel syndrome (Jeppesen et al. 2001), or be given GC for chronic medical condition such as asthma or Crohn's disease. It is not known if the administration of GLP-2 or DEX to the lactating mother will affect the lipid uptake of the offspring. Accordingly, this study was undertaken to determine 1) the influence of GLP-2, DEX, and GLP-2 + DEX, when administered to lactating rat dams, on the *in vitro* intestinal uptake of lipids in the suckling and weanling offspring; 2) if these changes in lipid uptake are due to variations in the intestinal morphology or mass, or to alterations in the abundance of selected cytosolic lipid binding proteins in the enterocytes; and 3) if GLP-2, DEX, or GLP-2 + DEX given to the lactating dams have a late effect on lipid uptake in the offspring after weaning.

4.2 MATERIALS AND METHODS

4.2.1. Animals

The principles for the care and use of laboratory animals, approved by the Canadian Council on Animal Care and by the Council of the American Physiological Society, were observed in the conduct of this study. All experiments were approved by the Animal Ethics Board, University of Alberta. Eight Sprague Dawley rats two weeks pregnant were obtained from Bio Science Animal Services, University of Alberta.

The dams were randomized into four groups which received treatment with GLP-2, DEX, GLP-2 plus DEX, or placebo. The treatment was started after delivery and was continued until the offspring were 19-21 days old. GLP-2 was administrated in a dose of 0.1 μg/g body weight/day subcutaneously [sc] twice per day at 7am and 7 pm. DEX was administrated in a dose of 0.128 μg/g body weight/day sc once per day at 7 pm. The regimen used for GLP-2 + DEX group was GLP-2 0.1 μg/g body weight/day sc twice per day at 7 am and 7 pm plus DEX 0.128 μg/g body weight/day sc once per day at 7 pm. The placebo group received 0.9% saline in a volume equal with the volume of GLP-2 administrated daily per rat (depending on the weight of the nursing rat, the volume ranged from 0.46 ml to 0.50ml) sc twice per day at 7 am and 7 pm.

The offspring were downsized after delivery to 12 pups, which were housed with their dams. There were 2 dams and 24 pups in each group. At weaning, eight offspring per group ("sucklings") were sacrificed for the uptake studies and eight per group were sacrificed for morphology and immunohistochemistry. The remaining post-weaning animals ("weanlings"), were sacrificed for uptake studies at 7 weeks of age [Figure 8].

The animals were housed at a temperature of 21°C, and in each day they were exposed to 12 hours of light and 12 hours of darkness. During the suckling period the offspring received only the dam's milk because the food was too far to be reached by the small rats. The weanlings were housed in pairs. Their water and food were supplied *ad libitum*. The dams and the weanlings were fed standard rat chow, PMI # 5001 (Nutrition International LLC, Brentwood, MO, USA). The diets were nutritionally adequate, providing for all known essential nutrient requirements. Body weights were recorded at the time of weanling and then weekly for the next four weeks.

4.2.2. Uptake Studies

Probe and marker compounds

The [^{14}C]-labeled probes included cholesterol (0.05 mM) and six fatty acids (0.1 mM): lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The labeled and unlabeled probes were supplied by Amersham Biosciences Inc. (Baie d'Urfe, PQ). The lipid probes were prepared by solubilizing them in 10 mM taurodeoxycholic acid (Sigma Co., St Louis, MO) in Krebs-bicarbonate buffer, with the exception of 12:0 which was solubilized only in Krebs-bicarbonate buffer. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume (Westergaard and Dietschy 1974).

Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl® (sodium pentobarbital, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed and rinsed with 150 ml cold saline. The intestine was divided into two parts: the proximal half of the intestine beginning at the ligament of Treitz was termed the "jejunum", and the distal half was termed the "ileum". A 2 cm piece of each segment of jejunum and ileum was gently scraped with a glass slide. The mucosal scrapings and the remaining wall of the intestine were dried overnight in an oven at 55°C. The percentage of the intestinal wall comprised of mucosa was calculated. The remaining intestine was everted, and cut into small rings of approximately 2-4 mm each. These intestinal rings were immersed in pre-incubation beakers containing Krebs's buffer (pH 7.2) at 37°C, bubbled with oxygen plus bicarbonate (O_2-CO_2, 95:5 by volume), and were allowed to equilibrate for 5 minutes (Perin et al. 1997). Uptake was initiated by the timed transfer of the tissue rings from the pre-incubation buffer to a 5 ml plastic vial containing [^{3}H]-inulin and [^{14}C]-labelled lipids in Kreb's buffer bubbled with oxygen plus bicarbonate that had been equilibrated to 37°C in a shaking water bath. The intestinal rings were incubated in the lipid substrates for 5 minutes.

Determination of uptake rates

The rate of uptake of lipid was terminated by pouring the vial contents onto filters on an Amicon vacuum filtration manifold that was maintained under suction, followed by washing the intestinal rings three times with ice-cold saline. The tissue rings were placed on a glass slide, and were dried overnight in an oven at a constant temperature of 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M NaOH, scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes (Westergaard and Dietschy 1974). The rates of lipid uptake were determined as nmol of substrate absorbed per 100 mg dry weight of the whole intestinal wall per minute (Jd, nmol •100 mg tissue $^{-1}$• min $^{-1}$), or as nmol of substrate absorbed per 100 mg dry weight of the mucosa per minute (Jm, nmol•100 mg mucosal tissue $^{-1}$• min $^{-1}$).

4.2.3. Morphological analysis

In order to determine the morphological characteristics of the intestine, a vertical section was prepared from the jejunum and from the ileum. Hematoxylin and eosin-stained slides were prepared from paraffin blocks. Crypt depth, villous height, villous width, villous width at half height, and cell density were measured using the program MetaMorph 5.05r (Universal Imaging Corporation, Downingtown, PA, USA). The group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of four animals in each group.

4.2.4. Immunohistochemistry

Jejunal and ileal tissues were embedded in paraffin, and 4-5 micron sections were mounted on glass slides. The sections were heated and placed immediately in xylene (2 x for 5 min each), followed by absolute ethanol (2 x for 2 min each), and were then rinsed with tap water. The slides were incubated in a hydrogen peroxide/methanol solution, and rinsed with tap water. Then they were rehydrated, and the tissue was encircled on the slides with a hydrophobic slide marker (PAP pen, BioGenex, California). The slides were incubated for 15 min in blocking reagent (20% normal goat serum) followed by primary antibody to I-FABP or to L-FABP for 30 min. Both antibodies were a generous gift from Dr. LB Agellon, University of Alberta. The slides were incubated in LINK® and LABEL®, and with DAB® solution (BioGenex, San Ramon, California). The slides were then washed, stained in hematoxylin, dehydrated in absolute ethanol, and cleared in xylene. The slides were photographed using AxioCam MRc (Zeiss), and the area labeled with antibody was determined using Metamorph 5.05r. The results were expressed as a ratio of the area which was antibody-positive versus the total area. Statistical analyses were based on a minimum of four villi per animal, and four animals per group.

4.2.5. Statistical analyses

The results were expressed as mean ± standard error of the mean. The statistical significance of the differences between the four groups was determined by analysis of variance (ANOVA) for values of $p \leq 0.05$. The significant differences between sucklings versus weanlings were determined using a Student's t-test. Statistical significance was accepted for values of $p \leq 0.05$.

4.3 RESULTS

4.3.1. Body and Intestinal Weights, and Villous Morphology

When the lactating dams were given GLP-2 or DEX, the mean body weight of the sucklings was less than in controls, and this decline was prevented with the combination of GLP-2 + DEX (Table 20). There was no difference in the body weights of weanling rats whose lactating dams had preciously been given GLP-2, DEX, or GLP-2 + DEX.

In suckling animals whose lactating dams were given GLP-2, there was no change in the characteristics of the jejunum or ileum (Table 21). When DEX was given, there was an approximately 50% fall in the weight of the jejunal wall, a 70% decline in the jejunal mucosal weight, and a 40% decline in the percentage of the intestinal wall comprised of mucosa. DEX had no effect on the characteristics of the ileum. When GLP-2 was added to DEX (GLP-2 + DEX), the marked loss in jejunal mass observed with DEX alone was not observed.

In weanling rats whose lactating dams were given GLP-2, the weight of the jejunal mucosa was greater than those given placebo (Table 22). DEX had no effect on the intestinal characteristics. GLP-2 + DEX markedly increased the weight of the jejunal submucosa, and decreased the percentage of the jejunal wall comprised of mucosa.

GLP-2 had no effect on the ileal morphology in sucklings or on the jejunal or ileal morphology of the weanlings (Table 23-26). In contrast, in the jejunum of sucklings GLP-2 increased villous height, width, crypt depth, distance between villi, and distance between enterocytes. DEX increased jejunal villous width, distance between villi, and distance between enterocytes in sucklings. In contrast, in weanlings DEX was associated with a decline in all of these endpoints. GLP-2 + DEX had no effect on jejunal or ileal morphology in sucklings, but in weanlings GLP-2 + DEX was associated with a decline in all of the jejunal and ileal morphological parameters.

4.3.2. Lipid uptake

Because of the influence of the treatments on the intestinal characteristics and morphology (Tables 21-26), the rate of lipid uptake was expressed on the basis of the weight of the mucosa (Jm, mmol/100mg mucosa $^{-1}$ /minute $^{-1}$), as well as on the basis of the weight of the intestinal wall (Jd, mmol/100mg $^{-1}$/min $^{-1}$).

When the lactating mothers were treated with GLP-2, there was no change in the jejunal uptake (Jd and Jm) of lipids in suckling rats, whereas the ileal uptake (Jm) of 16:0 was increased and the ileal uptake (Jm) of 18:2 was decreased (Tables 27 and 28). DEX increased the jejunal uptake (Jd and/or Jm) of 12:0, 18:0, 18:1, 18:3 and cholesterol, and increased the ileal uptake (Jd) of 16:0 (Tables 27 and 28). GLP-2 + DEX increased the jejunal and ileal uptake (Jd and/or Jm) of 12:0, 16:0, 18:0 and 18:1 as well as the jejunal uptake of 18:3 and cholesterol (Tables 27 and 28).

When uptake was examined in post-weaning rats whose dams were exposed to GLP-2 one month previously during lactation, there was reduced jejunal and ileal uptake

(Jd and/or Jm) of 12:0, 16:0 and 18:1, as well as increased jejunal uptake of cholesterol and ileal uptake of 18:2 and cholesterol (Tables 29 and 30). DEX reduced the jejunal uptake (Jd and Jm) of 18:1, and had no effect on ileal uptake. The combination of GLP-2 + DEX reduced the jejunal uptake (Jd and/or Jm) of 16:0 and 18:1, and increased the jejunal uptake (Jd) of 18:2. In the ileum of weanlings, GLP-2 + DEX reduced the uptake (Jd and Jm) of 18:1, and increased the uptake (Jd and Jm) of 18:2 (Tables 29 and 30).

The jejunal uptake (Jd and/or Jm) of 18:2 and 18:3 was lower in weanlings than in suckling rats given placebo (Tables 31 and 32). In controls, the ileal uptake (Jd and/or Jm) of 12:0, 18:0, 18:2, 18:3 and cholesterol was lower in weanlings than in sucklings (Tables 33 and 34). In GLP-2 rats, the jejunal uptake (Jd and/or Jm) of 12:0, 16:0 and 18:1 was lower in weanlings than in sucklings. In the ileum of GLP-2, the uptake (Jd and/or Jm) of all fatty acids was lower in weanlings than in sucklings. The jejunal and ileal uptake (Jd and/or Jm) of most fatty acids was lower in weanlings than in sucklings given DEX, and the same pattern persisted for rats given GLP-2 + DEX. Thus, lipid uptake falls with aging, and this process is not altered by exposure of the mother to GLP-2, DEX, or GLP-2 + DEX.

4.3.3. Immunohistochemistry

In sucklings, neither GLP-2 nor DEX had an effect on the jejunal or ileal abundance of I-FABP or L-FABP, whereas the ileal abundance of I- and L-FABP was increased in GLP-2 + DEX (Table 35).

4.4 DISCUSSION

Sucklings

GLP-2 given to lactating rats for 19-20 days in a dose of 0.1 μg/g body weight had no consistent effects on lipid uptake in either the jejunum or the ileum of the suckling offspring (Tables 27 and 28). These results were not expected, because it is known that in adult mice, the administration of GLP-2 at a dose of 5μg/day (approximately 0.16 μg/g body weight/day, slightly greater than our dose of 0.1 μg/g body weight/day) for 10 days increased the absorption of C^{14} triolein (Brubacker et al. 1997). Also surprisingly, the administration of GLP-2 to the lactating rat dams resulted in a decrease in body weight of the sucklings (Table 20). This is in contrast to the increase in the body weight of adults given GLP-2 (Scott et al. 1998, Jeppesen et al. 2001). GLP-2 is known as having a potent trophic effect on the intestine of adult animals (Drucker 1996, 2003). Also in this study, GLP-2 increased all the morphological parameters in the jejunum of sucklings.

It is unknown if GLP-2 crosses into mother's milk, although other hormones such as somatostatin do cross into milk (Grosvenor et al 1992, Goldman 2000). GLP-2 degradation in the intestinal lumen was not investigated, and it is possible that even if GLP-2 passed into mother's milk, that it might be sensitive to the intestinal proteases, and would possibly be destroyed (Rao et al. 1998, Fellah et al. 2001). Interestingly, the lack of effect of GLP-2 on lipid uptake is unlikely to be due to a lack of GLP-2R expression, because the level of GLP-2R mRNA is higher in fetal and neonatal rodent intestine than in adults (Burrin et al. 2000, Lovshin et al. 2000). Furthermore, there was evidence for a biological effect of GLP-2, such as its negative effect on body weight (Table 20), its effect on preventing the loss of jejunal mass with DEX (Table 21), its positive effects on jejunal morphology in sucklings (Table 23), its effects on the jejunal mucosa and submucosa in weanlings given GLP-2 or GLP-2 + DEX (Table 22), its enhancing effect on the ileal abundance of I- and L-FABP (Table 35), and its antiabsorptive effect on lipid uptake in weanlings (Tables 29 and 30). There are numerous peptides involved in the ontogeny of the intestine, such as hepatocyte growth factor, fibroblast growth factor, platelet-derived growth factor, and granulocyte colony-stimulating factor, and some of these peptides pass into milk (Calhoun et al. 1999, 2001, Grosvenor et al. 1993, Sukhotnik et al.2003). We speculate that GLP-2 either acts directly on the GLP-2 receptors in the intestine, or induces the release of other mediators which cross into mother's milk, and are themselves responsible for these numerous effects of GLP-2.

DEX treatment of lactating rats for 19-21 days in a dose of 0.128μg/g body weight per day increased lipid uptake in the jejunum of sucklings (Table 27 and 28). This confirms the lipid uptake enhancing effect of DEX shown in previous experiments with adult animals treated with GC for four weeks starting at three weeks of age (Thiesen et al. 2002, 2003). This enhanced uptake of lipids caused by DEX could be explained partially by a decline in the effective resistance of UWL, as demonstrated by an increased uptake of 12:0 (Westergaard and Dietschy 1974). There was no correlation between the increase in lipid uptake and the intestinal characteristics or the animal's body weights. In the suckling offspring, DEX treatment reduced the body weight as well as the weight of the jejunal wall and mucosa (Tables 20 and 21), although DEX resulted in an increase in villous height and width at the base, distance between villi, and enterocyte size (Table 23). This increase of distance between villi can explain why the characteristics of the intestine decreased, even though DEX increased the morphological parameters of the villi. The loss in body weight was anticipated, because DEX has catabolic effects on the whole body (Rooman et al. 1999, Goordon et al. 2001). Also, DEX induces atrophy of the intestine (Burrin et al. 1999, Park 1994) by decreasing enterocyte turnover (Batt and Scott 1982) and cell proliferation by blocking growth hormone signaling (Skouteris et al 1996, Croxtall et al 2000).

The enhancement in jejunal uptake of lipids with DEX occurred despite the lack of change in the enterocyte cytosolic I- and L-FABP (Table 35). Further data suggests that the alterations in lipid uptake were not due the I- and L-FABP: GLP-2 + DEX increased lipid uptake in the jejunum and ileum, but the abundance of I- and L-FABP in the jejunum did not change (Table 35). Previous studies in adult rats also failed to show a correlation between changes in lipid binding proteins and lipid uptake (Thiesen et al. 2002, Drozdowsky et al. 2003). In addition, a change in the abundance or a deficiency in I-FABP did not result in parallel alterations of intestinal lipid uptake (Agellon et al. 2002, Vassileva et al. 2000). Also, taking into account the complementary roles of L-FABP and ILBP, it has been suggested that L-FABP is not necessary for lipid uptake into enterocytes (Besnard et al. 2002).

Glucocorticosteroids such as DEX act on gene transcription by way of their effects on nuclear receptors (Bamberg et al. 1996, Sheepard 2002). DEX could potentially influence other lipid binding proteins not measured in this studies (such as caveolin-1, the SR-BI scavenger receptor, $FABP_{pm}$, FAT/CD36, FATP4, the cholesterol transport protein and ILBP), and thereby modify lipid uptake. The fluidity of BBM increases in fetal rats whose mothers received DEX (Neu et al. 1986) or in adult rats given DEX (Brasitus 1987), and this increase in BBM fluidity could possibly contribute to increased lipid uptake. We did not measure BBM fluidity in this study.

DEX crosses into mother's milk, and directly influences the intestine of the suckling offspring (Angelucci et al. 1985, Grosvenor et al. 1993, Pacha et al. 2000). The decrease in body weight of offspring and the atrophy of the mucosa are likely the result of DEX down-regulating the IGF-I hepatic endocrine axis, and inducing catabolism in the whole body as well as in the intestine (Kritsch et al. 2002). Interestingly, after DEX treatment, the IGF-I decline is greater in the wall of the intestine than in the villi (Gordon et al. 2001), and this may explain the decrease in intestinal characteristics (Table 21).

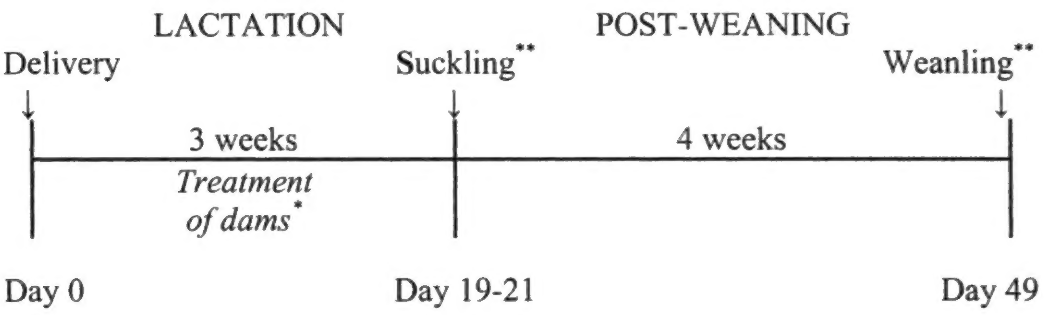
GLP-2 + DEX also increased lipid uptake in sucklings in both the jejunum and the ileum (Tables 27 and 28). Interestingly, this enhancement was similar to the effects of DEX in the jejunum. This enhancing effect of DEX or of GLP-2 + DEX could be partially due to a decrease in UWL (Tables 27 and 28). The mucosal mass was decreased in DEX, and was restored to normal in GLP-2 + DEX. Thus, alterations in the mucosal mass or morphology are not the cause of the increased uptake of lipid in GLP-2 + DEX (Tables 21, 23 and 24).

Weanlings

The jejunal and ileal uptake of lipids was lower in weanlings than in sucklings (Tables 31-34). A similar age-dependent decline in lipid absorption was demonstrated by Frost (1983) and by Flores (1989), measuring the absorption of C^{14}-labelled substrates. This age-associated fall in lipid uptake may be due a decline in the fluidity of the BBM (Schwarz et al. 1985, Hubner et al. 1988). The content of lipids in the lumen influences lipid uptake, so the age-associated decline in lipid uptake may be the result of the switch from the high-lipid milk diet consumed by sucklings to the high-carbohydrate diet which is eaten at weaning (Shiau 1987, Pacha 2000).

This decline in lipid uptake in early life appears to be a process that is not affected by GLP-2, DEX or GLP-2 + DEX (Tables 31-34). In contrast to the relative lack of effect of GLP-2 on lipid uptake in sucklings (Tables 27 and 28), one month after the administration of GLP-2 to the lactating dams, there was now reduced jejunal and ileal uptake (Jd and /or Jm) of lipids (Table 29 and 30). Furthermore, the stimulating effect of DEX on lipid uptake in suckling was lost in weanlings, but GLP-2 + DEX resulted in a mixed picture in weanlings, with the increase in the uptake of some lipids and a decrease in others (Tables 29 and 30). We did not establish the mechanisms of these late effects of GLP-2, DEX, or GLP-2 + DEX on lipid uptake. It is known that there are late effects of early nutrition on the absorptive function of the small intestine (Ferraris and Diamond 1989). Also, variations of the type of lipids in the diet of pregnant or lactating mothers modify the normal ontogeny of intestinal absorption (Thomson et al. 1989, Keelan et al. 1990, Perin et al. 1999). This late appearance of an effect of GLP-2 in weanlings raises the possibility that the GLP-2 given to lactating dams resulted in some alterations which led to a change in the ontogeny of lipid absorption in the intestine. Some mothers might require GC to be given for health reasons during lactation. The catabolic effects of GC on body weight and on the intestine of the offspring could be reversed by giving GLP-2 with the GC. However, this potential benefit needs to be weight against the possible adverse effect of an increase in two of the lipid binding proteins in the intestine, and the mixed effect of GLP-2 + DEX on lipid uptake. The late enhancement in I-and L-FABPexpressionand in the uptake of some lipids a month after the lactating mothers were given GLP-2 + DEX raises the possibility that the abnormal intestinal handling of lipids may continue in later life, and thereby contribute to abnormalities in lipid metabolism, which could be detrimental to the welfare of the animal.

FIGURE 8. Experiment 2 design


LACTATION
POST-WEANING
Delivery
Suckling**
Weanling**
3 weeks
4 weeks
*Treatment of dams**
Day 0
Day 19-21
Day 49

* Treatment with GLP-2, DEX, GLP-2 + DEX, and Placebo was administrated each day during lactation.

** Uptake studies were performed at day 19-21 ("suckling") and day 49("weanling").

Table 20. Body weights of suckling and weanling rats of dams injected through lactation with GLP-2, DEX, GLP-2 + DEX, or Placebo

	CONTROL	**GLP-2**	**DEX**	**GLP-2+ DEX**
SUCKLING	57.5 ± 2.0 a	49.1 ± 1.0 b	44.9 ± 1.2 b	54.3 ±1.5 a
WEANLING	272.3 ± 17.5	258.9± 13.6	240.3 ± 17.3	253.8 ± 13.6

Values are expressed mean ± sem. There were 8 rats in each group
a, b : values with different letters are significantly different $p < 0.05$ by ANOVA
The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling animals were sacrificed on day 19-21. There were 8 rats per treatment group.

Table 21. Intestinal characteristics of suckling rats of dams injected through lactation with GLP-2, DEX, GLP-2 + DEX, or Placebo

		CONTROL	GLP-2	DEX	GLP-2+DEX
JEJUNUM	Mucosa (mg/cm)	6.0 ± 0.5 a	5.0 ± 0.4 a	1.8 ± 0.3 b	5.9 ± 0.7 a
	Submucosa (mg/cm)	1.2 ± 0.2 a	1.1 ± 0.2 a	2.2 ± 0.3 b	0.9 ± 0.2 a
	Total Weight (mg/cm)	7.2 ± 0.5 a	6.1 ± 0.3 a	4.0 ± 0.5 b	6.8 ± 0.6 a
	% Mucosa	83.6 ± 2.4 a	81.0 ± 3.2 a	45.4 ± 5.1 b	85.1 ± 3.8 a
ILEUM	Mucosa (mg/cm)	2.5 ± 0.3	2.9 ± 0.4	2.5 ± 0.5	2.7 ± 0.2
	Submucosa (mg/cm)	1.2 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	1.1 ± 0.1
	Total Weight (mg/cm)	3.6 ± 0.3	4.3 ± 0.4	3.5 ± 0.4	3.8 ± 0.3
	% Mucosa	67.8 ± 4.2	66.2 ± 4.1	68.3 ± 7.8	70.4 ± 2.8

Values are expressed mean ± sem.
a, b : values with different letters are significantly different $p < 0.05$ by ANOVA. The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling animals were sacrificed on day 19-21. There were 8 suckling rats per treatment group.

Table 22. Intestinal characteristics of weanling rats of dams injected through lactation with GLP-2, DEX, GLP-2 + DEX, or Placebo

		CONTROL	GLP-2	DEX	GLP-2+DEX
JEJUNUM	Mucosa (mg/cm)	9.4 ± 0.8 a	11.9 ± 0.6 b	8.2 ± 1.0 a	7.2 ± 1.1 a
	Submucosa (mg/cm)	1.0 ± 0.1 a	0.8 ± 0.1 a	1.3 ± 0.2 a	4.0 ± 1.6 b
	Total Weight (mg/cm)	10.4 ± 0.8	12.7 ± 0.6	9.5 ± 0.9	11.1 ± 2.0
	% Mucosa	89.5 ± 1.3 a	93.6 ± 1.0 a	85.3 ± 2.1 a	69.0 ± 7.3 b
ILEUM	Mucosa (mg/cm)	8.8 ± 1.0	9.4 ± 0.6	8.4 ± 1.0	8.1 ± 0.9
	Submucosa (mg/cm)	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.2	0.9 ± 0.1
	Total Weight (mg/cm)	9.6 ± 0.9	10.3 ± 0.6	9.2 ± 0.9	9.0 ± 0.9
	% Mucosa	90.5 ± 2.0	90.4 ± 1.6	89.6 ± 2.4	89.7 ± 1.4

Values are expressed mean ± sem. a, b : values with different letters are significantly different $p < 0.05$ by ANOVA. The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The weanling animals were sacrificed on day 49. There were 8 weanling rats per group treatment.

Table 23. The effect of treatment of lactating rat dams with GLP-2, DEX and GLP-2 + DEX on jejunal morphology of the suckling offspring

Jejunum (µm)	**CONTROL**			**GLP2**			**DEX**			**GLP2+DEX**		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	353.8	±	28.4**a**	666.5	±	38.3**c**	544.8	±	39.8**bc**	416.7	±	64.8**ab**
Villous Width (base)	78.5	±	7.0**a**	171.4	±	4.6**c**	129.4	±	4.0**b**	110.7	±	19.3**ab**
Villous Width (half height)	79.1	±	3.2**a**	136.6	±	9.9**b**	99.8	±	4.6**a**	92.4	±	11.8**a**
Crypt Depth	58.2	±	6.1**a**	140.4	±	9.8**b**	83.7	±	1.8**a**	72.6	±	8.8**a**
Distance /5 villi	488.6	±	38.1**a**	1048.7	±	42.1**c**	813.3	±	43.1**b**	570.9	±	98.7**a**
Distance /5 cells	29.5	±	1.1**a**	42.1	±	3.0**b**	46.7	±	1.0**b**	33.2	±	4.0**a**

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA.
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling rats were sacrificed on day 19-21. In each group were 8 suckling rats.

Table 24. The effect of treatment of lactating rat dams with GLP-2, DEX and GLP-2 + DEX on ileal morphology of the suckling offspring

Ileum (µm)	**CONTROL**			**GLP2**			**DEX**			**GLP2+DEX**		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	240.4	±	25.0	238.2	±	17.8	231.2	±	74.1	269.5	±	48.7
Villous Width (base)	72.1	±	3.8	81.3	±	7.8	77.0	±	17.8	97.4	±	9.1
Villous Width (half height)	65.4	±	4.5	67.0	±	16.9	58.0	±	15.1	89.8	±	13.6
Crypt Depth	56.3	±	9.3	49.0	±	6.0	49.8	±	15.1	75.9	±	9.7
Distance /5 villi	443.9	±	19.8	596.1	±	79.8	529.8	±	104.4	718.1	±	88.1
Distance /5 cells	28.0	±	1.4	34.9	±	4.4	33.8	±	4.4	35.0	±	5.6

Values are mean ± sem
None of these differences was statistically significant tested by ANOVA
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling rats were sacrificed on day 19-21. In each group were 8 suckling rats.

Table 25. The effect of treatment of lactating rat dams with GLP-2, DEX and GLP-2 + DEX on jejunal morphology of the weanling offspring

Jejunum (µm)	**CONTROL**			**GLP2**			**DEX**			**GLP2+DEX**		
Weanling	**Mean**		**Sem**	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	727.1	±	44.9**a**	720.8	±	77.6**a**	392.8	±	44.2**b**	441.9	±	15.1**b**
Villous Width (base)	230.6	±	3.3**a**	240.8	±	7.3**a**	105.9	±	7.1**c**	154.5	±	16.7**b**
Villous Width (half height)	200.3	±	4.8**a**	187.3	±	7.2**a**	82.5	±	4.1**c**	117.9	±	17.3**b**
Crypt Depth	147.9	±	5.7**a**	150.6	±	11.9**a**	76.9	±	3.2**b**	114.2	±	14.7**a**
Distance /5 villi	1218.4	±	47.9**a**	1234.3	±	42.0**a**	565.2	±	21.4**c**	865.4	±	108.1**b**
Distance /5 cells	46.1	±	0.7**a**	44.4	±	0.7**a**	31.0	±	2.8**b**	40.1	±	4.2**a**

Values are expressed mean ± sem.
None of these differences was statistically significant tested by t-test
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The weanlings were sacrificed 28 days after weanling. In each group were 8 rats.

Table 26. The effect of treatment of lactating rat dams with GLP-2, DEX and GLP-2 + DEX on ileal morphology of the weanling offspring

Ileum (µm)	**CONTROL**			**GLP2**			**DEX**			**GLP2+DEX**		
Weanling	**Mean**		**Sem**	Mean		Sem	Mean		Sem	Mean		Sem
Villous height	583.6	±	27.2**a**	635.6	±	27.1**a**	280.7	±	8.1**b**	324.2	±	15.0**b**
Villous Width (base)	225.3	±	20.2**a**	223.3	±	19.8**a**	110.3	±	6.4**b**	114.1	±	8.2**b**
Villous Width (half height)	148.1	±	8.9**a**	159.0	±	14.3**a**	77.9	±	1.5**b**	79.0	±	5.9**b**
Crypt Depth	138.2	±	9.1**a**	168.0	±	3.8**a**	78.7	±	4.2**c**	70.6	±	5.2**c**
Distance /5 villi	1098.4	±	32.0**a**	1079.2	±	22.8**a**	526.8	±	21.7**b**	547.3	±	19.8**b**
Distance /5 cells	85.9	±	7.3**a**	69.8	±	7.0**a**	28.8	±	2.5**b**	35.8	±	1.3**b**

Values are expressed mean ± sem.
None of these differences was statistically significant tested by t-test
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The weanlings were sacrificed 28 days after the weanling. In each group were 8 rats.

Table 27. Effect of treatment of dams during lactation on jejunal and ileal uptake (Jd) lipids in suckling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	9.0±0.7 b	8.0±0.9 b	25.5±4.4 a	31.6±6.7 a
	16:0	1.7±0.3 b	2.4±0.3 ab	1.6±0.3 ab	3.0±0.4 a
	18:0	1.2±0.2 b	1.4±0.4 b	1.6±0.3 b	3.7±0.8 a
	18:1	1.5±0.3 b	1.0±0.1 b	1.9±0.2 b	3.5±0.7 a
	18:2	1.9±0.3	1.7±0.2	1.5±0.2	2.5±0.6
	18:3	2.0±0.3 b	1.6±0.2 b	1.6±0.2 b	3.2±0.6 a
	Chol	0.8±0.1 b	0.4 ±0.1 b	0.8±0.1 b	1.3±0.2 a
Ileum	12:0	14.1 ±0.6 b	17.2±1.6 b	18.7±2.6 b	49.0±6.0 a
	16:0	1.0±0.2 b	2.0±0.3 ab	2.0±0.4 a	2.0±0.2 a
	18:0	2.4±0.4 b	1.3±0.2 b	1.7±0.3 b	3.8±0.5 a
	18:1	1.0±0.3 b	1.5±0.2 b	1.2±0.2 b	2.5±0.3 a
	18:2	2.3±0.5 ab	1.0±0.1 b	1.6±0.4 a	2.9±0.3 a
	18:3	2.3±0.3 ab	1.1±0.1 b	2.2±0.4 a	2.7±0.4 a
	Chol	1.0±0.2 ab	0.6±0.1 b	0.9±0.2 ab	1.4±0.2 a

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling animals were sacrificed on day 19-21 and there were 8 suckling rats in each group.

Table 28. Effect of treatment of dams during lactation on jejunal and ileal uptake (Jm) of lipids in suckling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	14.7±1.7 c	9.9±1.1 c	64.3±12.4 a	38.2±9.3 b
	16:0	2.1±0.4	3.0±0.3	3.8±0.6	3.7±0.6
	18:0	1.7±0.2 b	1.6±0.4 b	4.3±1.0 a	4.4±0.9 a
	18:1	1.7±0.4 b	1.3±0.2 b	4.9±0.8 a	4.1±0.7 a
	18:2	2.3±0.3	2.1±0.3	3.7±0.5	2.6±0.6
	18:3	2.2±0.3 b	2.0±0.3 b	4.5±0.7 a	3.7±0.6 ab
	Chol	0.8±0.1 b	0.4±0.1 b	2.1±0.3 a	1.7±0.3 a
Ileum	12:0	20.3±2.5 b	27.0±3.0 b	31.6±6.2 b	63.0±8.0 a
	16:0	1.5±0.4 b	3.2±0.4 a	2.3±0.4 ab	2.9±0.3 a
	18:0	3.8±0.6 ab	2.2±0.4 b	2.9±0.6 b	5.2±0.7 a
	18:1	1.5±0.4 b	2,2±0.3 b	2.4±0.7 b	3.6±0.5 a
	18:2	3.9±0.8 a	1.6±0.2 b	2.7±0.7 a	3.6±0.5 ab
	18:3	3.6±0.5	1.7±0.2	3.6±0.8	3.7±0.5
	Chol	1.7±0.3	0.9±0.2	1.6±0.3	2.1±0.3

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The suckling animals were sacrificed on day 19-21 and there were 8 suckling rats in each group.

Table 29. Effect of treatment of dams during lactation on jejunal and ileal uptake (Jd) of lipids in weanling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	8.3±1.5 a	2.5±0.5 b	6.6±0.7 a	8.6±0.6 a
	16:0	1.4±0.2 a	0.8±0.1 b	1.1±0.2 a	1.3±0.2 b
	18:0	1.5±0.2	1.3±0.1	1.3±0.1	1.3±0.2
	18:1	1.7±0.2 a	1.3±0.1 a	0.8±0.1 b	0.6±0.2 b
	18:2	1.2±0.1 b	1.1±0.2 b	1.3±0.1 ab	1.7±0.2 a
	18:3	1.3±0.1	1.5±0.2	1.4±0.2	1.0±0.2
	Chol	0.6±0.1 b	1.4±0.3 a	1.0±0.1 b	1.0±0.1 b
Ileum	12:0	12.3±0.3 a	2.4±0.7 b	6.5±0.6 ab	7.0±0.8 ab
	16:0	1.0±0.1 a	0.5±0.1 b	0.6±0.1 ab	1.0±0.1 a
	18:0	1.0±0.1	1.3±0.1	0.8±0.1	0.7±0.1
	18:1	0.9±0.1 a	0.7±0.1 ab	0.8±0.1 a	0.4±0.1 b
	18:2	0.6±0.1 b	1.0±0.1 a	0.5±0.1 b	1.1±0.2 a
	18:3	0.7±0.1	0.7±0.1	0.8±0.1	0.8±0.1
	Chol	0.5±0.1 b	0.8±0.1 a	0.5±0.1 b	0.4±0.1 b

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The weanling animals were sacrificed 28 days after the weaning and there were 8 rats in each group.

Table 30. Effect of treatment of dams during lactation on jejunal and ileal uptake (Jm) of lipids in weanling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	10.2±1.7 a	2.1±0.5 b	7.4±0.7 ab	12.9±1.1 a
	16:0	1.5±0.2 a	0.8±0.1 b	1.3±0.2 a	1.8 ±0.2 a
	18:0	1.7±0.2	1.3±0.1	1.6±0.1	2.1±0.4
	18:1	1.9±0.2 a	1.3±0.1 b	1.1±0.2 b	0.9±0.2 b
	18:2	1.4±0.1 b	1.1±0.2 b	1.6±0.2 b	3.0±0.3 a
	18:3	1.4±0.1	1.5±0.2	1.4±0.2	1.7±0.3
	Chol	0.7±0.1	1.3±0.2	0.9±0.1	1.0±0.1
Ileum	12:0	13.6±1.4 a	1.9±0.8 b	7.3±0.7 ab	6.9±0.7 ab
	16:0	1.0±0.1	0.4±0.1	0.7±0.4	0.9±0.1
	18:0	1.1±0.1	1.3±0.1	1.0±0.1	0.8±0.1
	18:1	1.0±0.1 a	0.6±0.1 b	1.0±0.1 a	0.4±0.1 b
	18:2	0.7±0.1 b	1.0±0.1 a	0.6±0.1 b	1.2±0.2 a
	18:3	0.8±0.1	0.8±0.1	0.8±0.1	1.0±0.2
	Chol	0.6±0.1 b	0.9±0.1 a	0.6±0.1 b	0.5±0.1 b

Values are mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The weanling animals were sacrificed 28 days after the weaning and there were 8 rats in each group.

Table 31. Comparison of lipid uptake (Jd) into the jejunum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	9.0±0.7	8.3±1.5	8.0±0.9	2.5±0.5#	25.5±4.4	6.6±0.7#	31.6±6.7	8.6±0.6#
16:0	1.7±0.4	1.4±0.2	2.4±0.3	0.8±0.1#	1.6±0.3	1.1±0.2	3.0±0.4	1.3±0.2#
18:0	1.2±0.2	1.5±0.2	1.4±0.4	1.3±0.1	1.6±0.3	1.3±0.1	3.7±0.8	1.3±0.2#
18:1	1.5±0.3	1.7±0.2	1.0±0.1	1.3±0.1	1.9±0.2	0.8±0.1#	3.5±0.7	0.6±0.2#
18:2	1.9±0.3	1.2±0.1#	1.7±0.2	1.1±0.2	1.5±0.2	1.3±0.1	2.5±0.6	1.7±0.2
18:3	2.0±0.3	1.3±0.1#	1.6±0.2	1.5±0.2	1.6±0.2	1.4±0.2	3.2±0.6	1.0±0.2#
Chol	0.8±0.1	0.6±0.1	0.4±0.1	1.4±0.3#	0.8±0.1	0.8±0.1	1.3±0.2	0.8±0.1#

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 32. Comparison of lipid uptake (Jm) into the jejunum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	10.8±0.9	10.2±1.7	9.9±1.1	2.1±0.5#	64.3±12.4	7.3±0.7#	38.1±9.3	12.9±1.1#
16:0	2.0±0.4	1.5±0.2	3.0±0.3	0.8±0.1#	3.8±0.6	1.3±0.2#	3.7±0.6	1.7±0.2#
18:0	1.6±0.2	1.7±0.2	1.6±0.4	1.3±0.1	4.3±1.0	1.6±0.1#	4.4±0.9	2.1±0.4#
18:1	1.6±0.4	1.9±0.2	1.3±0.2	1.3±0.1	4.9±0.8	1.1±0.2#	4.1±0.7	0.9±0.2#
18:2	2.3±0.3	1.4±0.1#	2.1±0.3	1.1±0.2#	3.7±0.5	1.6±0.2#	2.6±0.6	3.0±0.3#
18:3	1.8±0.3	1.4±0.1	2.0±0.3	1.5±0.2	4.5±0.7	1.4±0.2#	3.7±0.6	1.7±0.3#
Chol	0.8±0.1	0.7±0.1	0.4±0.1	1.3±0.2#	2.1±0.3	0.8±0.1#	1.7±0.3	1.0±0.1#

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 33. Comparison of lipid uptake (Jd) into the ileum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	14.1±0.6	12.3±0.2#	17.2±1.6	2.4±0.7#	18.7±2.6	6.5±0.6#	49.0±6.0	7.0±0.8#
16:0	1.0±0.2	0.9±0.1	2.0±0.3	0.5±0.1#	2.0±0.4	0.6±0.1#	2.1±0.2	0.8±0.1#
18:0	2.4±0.4	1.0±0.1#	1.3±0.2	1.3±0.1	1.7±0.3	0.8±0.1#	3.8±0.5	0.7±0.1#
18:1	1.0±0.3	0.9±0.1	1.5±0.2	0.7±0.1#	1.2±0.2	0.8±0.1	2.5±0.3	0.4±0.1#
18:2	2.3±0.5	0.6±0.1#	0.9±0.1	1.0±0.1	1.6±0.3	0.5±0.1#	2.9±0.3	1.1±0.2#
18:3	2.2±0.3	0.7±0.1#	1.1±0.1	0.7±0.1#	2.2±0.4	0.7±0.1#	2.7±0.4	0.8±0.1#
Chol	1.0±0.2	0.5±0.1#	0.6±0.1	0.8±0.1	0.8±0.2	0.5±0.1#	1.4±0.2	0.4±0.1#

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 34. Comparison of lipid uptake (Jm) into the ileum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	18.8±2.5	13.6±1.4	27.0±3.0	1.9±0.8#	31.6±6.2	7.3±0.7#	62.1±8.0	6.3±0.7#
16:0	1.4±0.3	1.0±0.1	3.2±0.4	0.4±0.1#	2.3±0.4	0.7±0.4#	2.9±0.3	0.9±0.1#
18:0	3.8±0.6	1.1±0.1#	2.2±0.4	1.3±0.1#	2.9±0.6	1.0±0.1#	5.2±0.7	0.8±0.1#
18:1	1.5±0.4	1.0±0.1	2.2±0.3	0.6±0.1#	2.4±0.7	1.0±0.1	3.6±0.5	0.4±0.1#
18:2	3.9±0.8	0.7±0.1#	1.6±0.2	1.0±0.1#	2.7±0.7	0.5±0.1#	3.6±0.5	1.2±0.2#
18:3	3.6±0.5	0.8±0.1#	1.7±0.2	0.8±0.1#	3.6±0.8	0.8±0.1#	3.7±0.5	1.0±0.2#
Chol	1.7±0.3	0.6±0.1#	0.9±0.2	0.9±0.1	1.6±0.3	0.6±0.1#	2.1±0.3	0.5±0.1#

Values are expressed as mean ± sem.

#: significantly different by t-test, $p<0.05$ sucklings versus weanlings

Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 35. The abundance of I-FABP and L-FABP in the intestine of suckling offspring whose mothers received treatment during lactation

		CONTROL	GLP2	DEX	GLP2+DEX
I_FABP	Jejunum	57.6 ± 2.6	56.9 ± 3.0	64.3 ± 1.3	63.3 ± 0.8
	Ileum	68.3 ± 2.9a	67.8 ± 1.4a	68.4 ± 3.2a	80.9 ± 1.5b
L_FABP	Jejunum	65.8 ± 1.5	67.9 ± 4.4	66.0 ± 1.6	68.7 ± 1.2
	Ileum	64.0 ± 2.4a	61.2 ± 0.6a	63.2 ± 1.5a	72.2 ± 1.9b

Values are ratio of area stained with antibody versus total tissue area and are expressed as mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during lactation. The sucklings were sacrificed on day 19-21. In each group were 8 suckling rats.

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CHAPTER 5

THE ILEAL MALABSORPTION OF LIPIDS IN SUCKLING RATS WHOSE PREGNANT AND LACTATING DAMS WERE GIVEN GLP-2 AND DEXAMETHASONE PERSISTS AFTER WEANING

5.1 INTRODUCTION

The ontogeny of the intestinal tract includes all the events involved in the development and maturation of the gut. This complex process involves the morphological maturation with the transition from the endodermal tube to the villous-crypt architecture, the functional maturation of the digestive and absorptive functions, as well as the barrier proprieties of the mucosa (Henning et al. 1987, 1994, Paulsen et al. 2003). The digestive functions exhibit age-dependent alterations in the absorption of nutrients during the suckling and weanling period (Sanderson and Walkers 2000). These variations are due to alterations of the abundance and/or activity of the transporters and digestive enzymes, as well as to changes in the permeability of BBM (Henning et al. 1994, Sanderson and Walkers 2000, Buddington and Malo 2003, Nanthakumar et al. 2003).

Intestinal lipid uptake involves both passive diffusion and protein-mediated transport (Besnard et al. 2000). The uptake of lipids is higher in suckling as compared with adult rats, possibly due to their increased BBM permeability as well as the more efficient metabolism of fat (Frost et al. 1983, Flores et al. 1989). The ontogeny of lipid absorption has not been extensively studied. GLP-2 enhances the absorption of sugars in adult animals (Cheeseman et al. 1996, 1997, 1998), but it is not known if this peptide influences the absorption of lipids. In adult rats, GC increase the uptake of sugars and lipids (Thiesen et al. 2002, 2003). GC may need to be given for health reasons to pregnant and lactating mothers, and it is not known if this affects the lipid absorption in their offspring, if this effect continues after GC are stopped, nor is it known if the effects of GC can be modified by given GLP-2.

Accordingly, this study was undertaken to determine 1) the influence of GLP-2, DEX, and GLP-2 + DEX, when administered to pregnant and lactating rat dams, on the intestinal *in vitro* uptake of fatty acids and cholesterol in their suckling offspring; 2) if the alterations in the uptake of lipids is due to variations in the intestinal morphology or mass, or to changes in selected lipid binding proteins in the cytosol of the enterocytes; and 3) if the changes persist a month later in post-weaning animals.

5.2 MATERIALS AND METHODS

5.2.1. Animals

The principles for the care and use of laboratory animals, approved by the Canadian Council on Animal Care and by the Council of the American Physiological Society, were observed in the conduct of this study. All experiments were approved by the Animal Ethics Board, University of Alberta. Eight Sprague Dawley rats one week pregnant were obtained from Bio Science Animal Services, University of Alberta. The dams were randomized into four groups which received treatment with GLP-2, DEX, GLP-2 plus DEX, or placebo. The treatment was started 10 days before delivery and was continued until the animals were 19-21 days of age. DEX was administrated in a dose of 0.128 μg/g body weight/day sc once per day at 7 pm. GLP-2 was administrated in a dose of 0.1 μg/g body weight/day sc twice per day at 7am and 7 pm. The regimen used for DEX + GLP-2 group was DEX 0.128 μg/g body weight/day sc once per day at 7 pm plus GLP-2 0.1 μg/g body weight/day sc twice per day at 7am and 7 pm. The placebo group received 0.9% saline sc in a volume equal with the volume of GLP-2 administrated daily per rat (depending on the weight of the dams, the volume ranged from 0.32 ml to 0.46ml), twice per day at 7 am and 7 pm.

After delivery, the offspring were downsized to 12 pups, which were housed with their dams. There were 2 dams and 24 pups in each group. At weaning, eight offspring per group ("sucklings") were sacrificed for the uptake studies and eight per group were sacrificed for morphology and immunohistochemistry [Figure 9]. The remaining post-weaning animals, ("weanlings"), were sacrificed for uptake studies at 7 weeks of age.

The animals were housed at a temperature of 21°C, and in each day they were exposed to 12 hours of light and 12 hours of darkness. During the suckling period the offspring received only the dam's milk because the food was too far to be reached by the small rats. The weanlings were housed in pairs. Their water and food were supplied *ad libitum*. The dams and the weanlings were fed standard rat chow, PMI # 5001 (Nutrition International LLC, Brentwood, MO, USA). The diets were nutritionally adequate, providing for all known essential nutrient requirements. Body weights were recorded at the time of weaning and then weekly for the next four weeks.

5.2.2. Uptake Studies

Probe and marker compounds

The [^{14}C]-labelled probes included cholesterol (0.05 mM) and six fatty acids (0.1 mM): lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). The labeled and unlabeled probes were supplied by Amersham Biosciences Inc. (Baie d'Urfe, PQ). The lipid probes were prepared by solubilizing them in 10 mM taurodeoxycholic acid (Sigma Co., St Louis, MO) in Krebs-bicarbonate buffer, with the exception of 12:0 which was solubilized only in Krebs-bicarbonate buffer. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume (Westergaard and Dietschy 1974).

Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl® (sodium pentobarbital, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed and rinsed with 150 ml cold saline. The intestine was divided into two parts: the proximal half of the intestine beginning at the ligament of Treitz was termed the "jejunum", and the distal half was termed the "ileum". A 2 cm piece of each segment of jejunum and ileum was gently scraped with a glass slide. The mucosal scrapings and the remaining wall of the intestine were dried overnight in an oven at 55°C. The percentage of the intestinal wall comprised of mucosa was calculated. The remaining intestine was everted, and cut into small rings of approximately 2-4 mm each. These intestinal rings were immersed in pre-incubation beakers containing Krebs's buffer (pH 7.2) at 37°C, bubbled with oxygen plus bicarbonate (O_2-CO_2, 95:5 by volume), and were allowed to equilibrate for 5 minutes (Perin et al. 1997). Uptake was initiated by the timed transfer of the tissue rings from the pre-incubation buffer to a 5 ml plastic vial containing [^{3}H]-inulin and [^{14}C]-labelled lipids in Krebs buffer bubbled with oxygen plus bicarbonate that had been equilibrated to 37°C in a shaking water bath. The intestinal rings were incubated in the lipid substrates for 5 minutes.

Determination of uptake rates

The rate of uptake of lipid was terminated by pouring the vial contents onto filters on an Amicon vacuum filtration manifold that was maintained under suction, followed by washing the intestinal rings three times with ice-cold saline. The tissue rings were placed on a glass slide, and were dried overnight in an oven at a constant temperature of 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M NaOH, scintillation fluid was added, and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes (Westergaard and Dietschy 1974). The rates of lipid uptake were determined as nmol of substrate absorbed per 100 mg dry weight of the whole intestinal wall per minute (Jd, nmol $\bullet$100 mg tissue $^{-1}\bullet$ min $^{-1}$), or as nmol of substrate absorbed per 100 mg dry weight of the mucosa per minute (Jm, nmol$\bullet$100 mg mucosal tissue $^{-1}\bullet$ min $^{-1}$).

5.2.3. Morphological analysis

In order to determine the morphological characteristics of the intestine, a vertical section was prepared from the jejunum and from the ileum. Hematoxylin and eosin-stained slides were prepared from paraffin blocks. Crypt depth, villous height, villous width, villous width at half height, and cell density were measured using the program MetaMorph 5.05r (Universal Imaging Corporation, Downingtown, PA, USA). The group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of four animals in each group.

5.2.4. Immunohistochemistry

Jejunal and ileal tissues were embedded in paraffin, and 4-5 micron sections were mounted on glass slides. The sections were heated and placed immediately in xylene (2 x for 5 min each), followed by absolute ethanol (2 x for 2 min each), and were then rinsed with tap water. The slides were incubated in a hydrogen peroxide/methanol solution, and rinsed with tap water. Then they were rehydrated, and the tissue was encircled on the slides with a hydrophobic slide marker (PAP pen, BioGenex, California). The slides were incubated for 15 min in blocking reagent (20% normal goat serum) followed by primary antibody to I-FABP or to L-FABP for 30 min. Both antibodies were a generous gift from Dr. LB Agellon, University of Alberta. The slides were incubated in LINK® and LABEL®, and with DAB® solution (BioGenex, San Ramon, California). The slides were then washed, stained in hematoxylin, dehydrated in absolute ethanol, and cleared in xylene. The slides were photographed using AxioCam MRc (Zeiss), and the area labeled with antibody was determined using Metamorph 5.05r. The results were expressed as a ratio of the area which was antibody-positive versus the total area. Statistical analyses were based on a minimum of four villi per animal, and four animals per group.

5.2.5. Statistical analyses

The results were expressed as mean ± standard error of the mean. The statistical significance of the differences between the four groups was determined by analysis of variance (ANOVA) for values of $p \leq 0.05$. The significant differences between sucklings versus weanlings were determined using a Student's t-test. Statistical significance was accepted for values of $p \leq 0.05$.

5.3 RESULTS

5.3.1. Body and Intestinal Weights, and Villous Morphology

There was no significant difference in the body weight gain (grams/day) among the dams used in the control, GLP-2, DEX, or GLP-2 + DEX groups (data not shown). Also, there were no differences in the body weights of the suckling or weanling rats in these four groups.

In sucklings given DEX or GLP-2 + DEX as compared with controls, there was increased jejunal mucosal weight, and an increase in the percentage of the jejunal wall comprised of mucosa (Table 36). There were no changes in the ileum. GLP-2 by itself had no effect on the characteristics of the jejunum or ileum. In weanling animals, 89.8% of the weight of the intestinal wall was comprised of mucosa (Table 37), much higher than the approximately 50% seen in the suckling animals (Table 36). In weanlings, there was no effect of GLP-2, DEX, or GLP-2 + DEX on the weight of the jejunal or ileal mucosa, the weight of the intestine, or on the percentage of the intestinal wall comprised of mucosa.

In sucklings, giving GLP-2 to the rat dams resulted in larger enterocytes in the jejunum (Table 38), and higher villi in the ileum (Table 39). The weanling rats of dams exposed to GLP-2, DEX, or GLP-2 + DEX one month previously exhibited an increase in jejunal villous height, villous width, and crypt depth (Table 40). The morphology of the ileum in weanling rats showed no difference between treatment groups (Table 41).

5.3.2. Lipid Uptake

Because of the effect of DEX and GLP-2 + DEX on the jejunal weights and morphology of the suckling rats (Tables 36-39), the rate of uptake of lipids was expressed on the basis of mucosal mass (Jm, mmoles · 100 mg mucosa $^{-1}$ · min $^{-1}$), as well as on the basis of the weight of the full thickness of the intestine (Jd, mmol · 100 mg^{-1} · min^{-1}).

In sucklings, GLP-2 increased the jejunal uptake (Jd) of cholesterol, and reduced the ileal uptake (Jd and/or Jm) of 12:0, 18:1 and 18:2. DEX reduced the jejunal and ileal uptake (Jd and/or Jm) of 12:0, 18:0, 18:1 and 18:2 (Tables 42 and 43). The combination of GLP-2 + DEX also reduced the jejunal uptake (Jd and/or Jm) of 12:0 and 18:0, and reduced the ileal uptake (Jd and/or Jm) of 12:0, 18:0, 18:1, 18:2 and cholesterol (Tables 42 and 43).

In weanlings, GLP-2 had no effect on the jejunal uptake of lipids, but reduced the ileal uptake (Jd and/or Jm) of 12:0, 18:1, 18:2 and 18:3 (Tables 44 and 45). DEX had no effect on the jeunal uptake of lipids, but reduced the ileal uptake (Jd and/or Jm) of 12:0, 16:0, 18:1 and 18:3 (Tables 44 and 45). GLP-2 + DEX reduced the jejunal uptake (Jd and/or Jm) of 12:0, and reduced the ileal uptake of 12:0, 16:0, 18:1 and 18:3 (Tables 44 and 45).

The uptake (Jd and/or Jm) of lipids was lower in the jejunum and ileum of weanlings as compared with sucklings in control rats, or in those given GLP-2 or GLP-2 + DEX (Tables 46-49). In DEX, the jejunal uptake (Jd or Jm) of lipids was similar in sucklings and in weanlings, and was lower in the ileum of weanlings than sucklings.

5.3.3. Immunohistochemistry

There were no differences between sucklings and weanlings in the abundance of I-FABP or L-FABP in the jejunum or ileum of control animals (Table 50). GLP-2, DEX and GLP-2 + DEX had no effect on the abundance of I- or L-FABP in suckling or weanling rats, except for a lower abundance of I-FABP in the ileum of weanlings given GLP-2 + DEX (Tables 51).

5.4 DISCUSSION

The abundance of GLP-2R mRNA is higher in fetal and neonatal rodent intestine than in adults (Burrin et al. 2000, Lovshin et al. 2000). The role of GLP-2 on the intestine of neonatal rats has not been reported previously. Also, it is not known what is the influence of GLP-2 on lipid absorption in immature rats, although in adult mice there is a report of enhanced intestinal absorption of C^{14} triolein after GLP-2 injections in a dose of 5 μg/day (approximately 0.16 μg/g body weight/day, slightly greater than our dose of 0.1μg/g body weight/day) for 10 days (Brubaker et al. 1997). GC enhances the uptake of lipids in adult rats (Thiesen et al. 2002), but it is not known if the effects of GC are modified by GLP-2.

Because GLP-2 has a pro-absorptive effect in adult rats and in humans (Scott et al. 1998, Jeppesen et al. 2001), it was expected that at this dose of 0.1 μg/g body weight/day for 30 days, the lipid uptake would be enhanced. However, treatment of the pregnant and lactating dams with GLP-2 resulted in a reduction in the ileal uptake of long chain fatty acids in both the suckling and weanling offspring (Tables 42-44). The mechanisms of this unexpected decline in the ileal uptake of long-chain fatty acids with GLP-2 was partially due to the enhanced resistance of the intestinal UWL (Westergaard and Dietschy 1974), since the uptake of lauric acid (12:0) declined in both sucklings and weanlings.

In adult rats, GLP-2 has a trophic effect on the intestine (Drucker 1996, 2003). The tissue characteristics of sucklings given GLP-2 showed an increase in the size of the jejunal enterocytes and an increase in the height of the ileal villi (Tables 39 and 40), but the weight of the mucosa and the percentage of the intestinal wall comprised of mucosa did not change (Table 36 and 37). In weanlings, villous height, villous width, and crypt depth were higher than in controls (Table 40). Thus, GLP-2 given to the pregnant and lactating rats did have a trophic effect on the intestine of the offspring, and this effect lasted for a month after the injections were completed. It is possible that GLP-2 might exert its influence on suckling and weanling offspring by way of an increase in intestinal proliferation or a decrease in apoptosis. Indeed, in adults, GLP-2 has an anti-apoptotic (Burrin et al. 2000, Drucker 2003) and trophic effect (Drucker 1996, 2003). We did not specifically measure apoptosis or proliferation in suckling and weanling animals. Nonetheless, clearly the reduction in lipid uptake in sucklings and weanlings treated with GLP-2 could not be explained by changes in the animal's body weight or by enhancement of the intestinal weight.

The GLP-2 secreting 'L' cells are more abundant in the distal than in the proximal small intestine (Kiefer et al. 1999, Burrin et al. 2001). This raises the possibility that the highest intestinal tissue levels of GLP-2 are likely to be in the ileum. However, in adult rats the prominent trophic effects of systemically administrated GLP-2 are observed in the jejunum (Brubaker and Drucker 2002), and the receptors for GLP-2 (GLP-2R) are more abundant in the jejunum as compared with the ileum (Munroe et al. 1999). Thus, the lack of stimulating effect of GLP-2 on the jejunal uptake of lipids in suckling or weanling animals is unlikely to be on the basis of a lack of GLP-2R.

It is important to stress that in this study, GLP-2 was given to the pregnant and lactating mothers. It is not known if GLP-2 crosses the placenta or through the mammary gland into mother's milk. Furthermore, any GLP-2 that might come into the intestinal lumen with the mother's milk might possibly be digested before acting, although breast milk may delay this process (Rao et al. 1998, Fellah et al. 2001).

The inhibitory effect of GLP-2 on lipid uptake into the ileum may not necessarily be a direct effect of GLP-2, but rather some other mediator may be involved such as hepatocyte growth factor, fibroblast growth factor, platelet-derived growth factor, and granulocyte colony-stimulating factor (Calhoun et al. 1999, 2000, 2001, Sukhotnik et al. 2003). It is unknown if these trophic factors are released in the pup in response to the administration of GLP-2 to the mother, and this subsequent exposure of the pup via placental transfer or via milkj. We speculate that GLP-2 influences the secretion of one or more of these factors, which would then act as a second messenger to inhibit lipid uptake into the intestine of the young pup.

When budesonide or prednisone is given to rats for four weeks, starting when the animals are three weeks of age, there is enhanced uptake of lipids (Thiesen et al. 2002). In contrast, DEX reduced the uptake of fatty acids in the ileum of suckling and weanling rats (Tables 38-41). This effect likely occurs during pregnancy, since DEX given during lactation enhances lipid uptake in the offspring (unpublished observations, 2003). Thus, DEX given to the pregnant and lactating mothers has a different effect on intestinal uptake of lipids in suckling versus older animals. This decrease in lipid uptake into the ileum of suckling and weanling rats given DEX was associated in part with a greater resistance of the UWL, as reflected by the lower uptake of lauric acid (12:0) (Tables 42-45). This decline in lipid uptake with DEX was also associated with an increase in the mucosal mass in the jejunum of the sucklings (Tables 36 and 37), although changes in villous morphology were found only in jejunum of the weanlings (Tables 38-41). Thus, the inhibitory effects of DEX on lipid uptake were not due to atrophy of the intestine, unlike the situation in adult rats in which DEX induces atrophy in the intestine (Park et al. 1994, Kritsch et al. 2000). In fact, there was an increase in the mucosal weight and percent of the intestinal wall comprised of mucosa in the jejunum of animals whose mothers had been given DEX (Table 36). This may possibly represent a greater ease of removal of the scraped mucosa.

It had been anticipated that the suckling and weanling rats would lose weight, because DEX has a catabolic effect on the whole body when given to adult rats (Park et al. 1994, Rooman et al. 1999). However, DEX administration to pregnant and lactating mothers in a dose of 0.128 μg/g body weight per day for 30 days had no effect on the body weights of the offspring. Clearly, the effect of GC on the intestine of adult animals is different in the newborn. Furthermore, the malabsorption of lipids in the ileum was not sufficient to cause weight loss. This may have been because lipid absorption in the proximal intestine remained sufficient to maintain body weight, or because the absorption of other nutrients was enhanced in a compensatory manner.

The mechanism of the decline in lipid uptake with DEX as well as with GLP-2 could not be explained by alterations in the abundance of the two cytosolic fatty acid binding proteins which were measured, because there was no change in the abundance of I-FABP or L-FABP in suckling or weanling animals, although in the ileum of weanlings given GLP-2 + DEX there was a decline in the abundance of I-FABP (Tables 50 and 51). Others have also reported that changes in I-FABP do not necessarily mimic alterations in lipid uptake (Agellon et al. 2002, Vassileva et al. 2000). Of note, there are several other lipid binding proteins in the BBM or enterocyte cytosol (such as caveolin-1, the scavenger receptor class B type I [SR-BI], the plasma membrane-fatty acid binding protein [FABPpm], the fatty acid transporter [FAT/ CD36], the fatty acid transport protein-4 [FATP4], the cholesterol transport protein and the ileal lipid binding protein) (Smart et al. 1999, Schulthess et al. 2000, Thomson et al. 2003). It is possible that GLP-2, DEX, or GLP-2 + DEX could reduce the abundance of some of these lipid binding proteins, and thereby reduce lipid uptake.

It is likely that the effect of GC on the intestine of the offspring was a direct effect, because the DEX injected into the mother crosses the placenta (Munson 1995, Reece et al. 1995) and also passes into her milk (Angelluci et al. 1985, Grosvenor et al. 1992, Pacha et al. 2000). GC may influence intestinal absorption by way of reducing proliferation and enhancing apoptosis (Batt and Scott 1982, Park et al. 1994, Croxtall et al. 2000, 2002), as well as by modifying gene transcription through their effects on nuclear receptors (Bamberg et al. 1996, Sheepard 2002). GC also reduce the effects of trophic peptides such as hepatocyte growth factor and epidermal growth factor (Skouteris et al. 1996, Croxtall et al. 2000, 2002). The lack of effect of GC on intestinal lipid binding proteins in previous studies (Thiesen et al. 2002, Thiesen 2002), as well as DEX in this study (Table 51), does not eliminate the possibility that the inhibitory effect of DEX on lipid uptake may be the result of some modification on other lipid binding proteins which we did not measure.

The combination of GLP-2 plus DEX also reduced the uptake of long-chain fatty acids into the ileum, consistent with the effects of GLP-2 alone and DEX alone (Tables 42-45). Thus, the combination of GLP-2 plus DEX had a qualitatively similar effect on the uptake of long-chain fatty acids as the effects of GLP-2 alone or DEX alone. However, clearly was no obvious additive or synergistic effect. Again, this change in lipid uptake with GLP-2 + DEX could be explained in part by an increase in the resistance of the UWL (Tables 43-45), but was not explained by alterations in the intestinal characteristics or morphology (Tables 36-41). Treatment with GLP-2 + DEX decreased the abundance of I-FABP in the ileum of weanlings (Table 51). This might partially explain the decrease in lipid uptake found in weanling animals, but does not explain the decline in uptake in sucklings, or the decline in uptake in weanlings given GLP-2 alone or DEX alone. It is unclear what is the biological significance of the increased I-FABP which resulted with GLP-2 + DEX.

Under physiologic conditions, lipids are preferentially absorbed in the jejunum (Shiau 1987, Meddings 1988). In our experiment the decline in lipid uptake with GLP-2, DEX, GLP-2 +DEX was observed largely in the ileum. It is also recognized that the ileum is more adaptable than is the jejunum, for example in response to alterations in nutrient upload, or following intestinal resection (Thompson et al. 1999, Thompson and Ferguson 2000, Thomson et al. 2003). It is possible, therefore, that the distal small intestine is more able to responde to the effects of GLP-2, DEX and GLP-2+DEX due to the lower base-line levels of lipid uptake, or perhaps due to its greater adaptive responsiveness to the treatment effects.

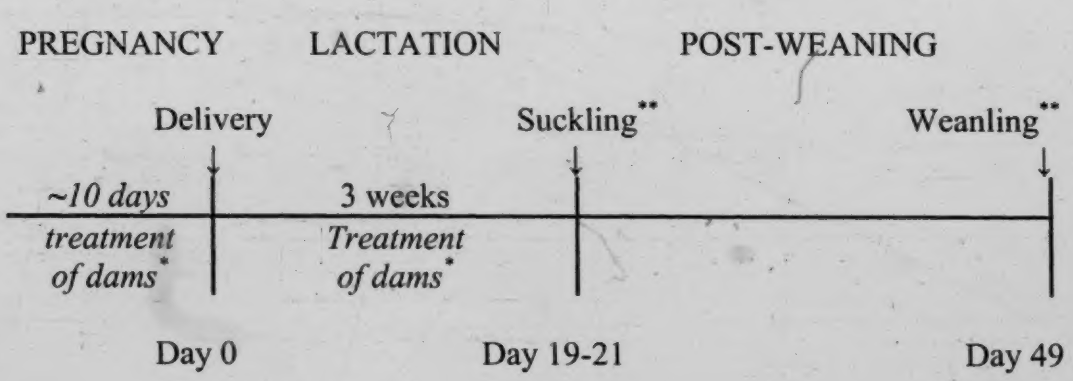
The GLP-2, DEX, or GLP-2 plus DEX were administered to the rat mothers during the last 10 days of their pregnancy, as well as during lactation up to day 19-21 in the suckling animals. In order to determine whether the effects of GLP-2, DEX, or the combination of GLP-2 plus DEX described above in the suckling rats had a persistent effect four weeks after the discontinuation of the injections, we first need to know what is the effect of age itself on lipid uptake. When comparing suckling with weanling rats given placebo (Tables 46-49), for both the jejunum and the ileum, there was a consistent decline in the uptake of long-chain fatty acids. Measuring the rate of triacylglycerol or C^{14} triolein entry into the plasma of suckling rats has shown a fall in the absorption of lipids with weaning (Frost et al. 1983, Flores et al. 1989). This fall is probably due to a lower permeability of BBM in adults as compared with sucklings, possibly as the result of a fall in BBM fluidity (Schwarz et al. 1985, Hubner et al. 1988). Also, this fall in lipid uptake between sucklings and adults may be the result of the usual decrease in the dietary content of fat and an increase in the content of carbohydrates as a result of the animals weaning from milk to a higher carbohydrate-containing diet. Luminal lipids may be important to set the level of fat absorption (Thomson et al. 1987). For example, in intestinal implants under the kidney capsule, the esterification activity of fatty acids is significantly reduced, which demonstrates that the developmental and adaptive changes of lipid absorption are primary modulated by the luminal lipids (Shiau 1987, Pacha 2000).

This age-associated decline in lipid uptake between suckling and weanlings was also observed in animals given GLP-2 or GLP-2 plus DEX. In contrast, in animals given DEX, the age-associated decline in lipid uptake into the jejunum was not observed (Table 46-49). It is unclear what are the mechanisms by which DEX prevented the age-associated decline in the uptake of long-chain fatty acids into the jejunum. This may be by the same process that permits DEX to stimulate lipid uptake in adult rats (Thiesen et al. 2002), or may be by a process which modifies the ontogeny of the lipid uptake.

The results found in weanlings appear to be late effects of GLP-2, DEX, and GLP-2 + DEX, because the effects persist four weeks after the injections with these agents were discontinued (Tables 42-45). It has long been appreciated that there are late effects of early nutrition (Ferraris and Diamond 1989). For example, changing the type of lipids in the diet of pregnant or lactating mothers modifies the normal ontogeny of intestinal absorption (Thomson et al. 1989, Keelan et al. 1990, Perin et al. 1999). The late effects of GLP-2 or DEX in our experiment could be the result of a re-programming effect of the normal ontogenic process related to lipid uptake. This alteration raises the alarming possibility that the administration of GLP-2 or DEX to the mother during pregnancy and lactation may have a lasting effect on lipid uptake in the offspring, both in sucklings and in weanlings. It is unknown how long this lipid malabsorption lasts, or whether there are other lasting and deleterious effects on lipid metabolism.

In summary, GLP-2, DEX, and GLP-2 plus DEX given to pregnant and lactating rat dams reduce the ileal uptake of long-chain fatty acids in suckling and weanling offspring (Table 42-45). This found malabsorption of lipid is not due to alterations in the body weight, in the characteristics of the intestine including villous morphology, or in the abundance of two enterocyte lipid binding proteins. This malabsorption of lipids persists long after the treatments have been stopped, and it is not known what might be the importance of this effect of GLP-2 or DEX on lipid malabsorption on the nutritional welfare of the animal in later life.

Figure 9. Experiment 3 design


PREGNANCY
LACTATION
POST-WEANING
Delivery
Suckling**
Weanling**
~10 days
treatment of dams*
3 weeks
Treatment of dams*
Day 0
Day 19-21
Day 49

*Treatment with GLP-2, DEX, GLP-2 + DEX, and Placebo was administrated during pregnancy and lactation.

**Uptake studies were performed at day 19-21 ("suckling") and day 49 ("weanling").

Table 36. Intestinal characteristics of suckling rats of dams injected through pregnancy and lactation with GLP-2, DEX, or GLP-2 + DEX

		CONTROL	GLP-2	DEX	GLP-2+DEX
JEJUNUM	Mucosa (mg/cm)	1.8 ± 0.3 a	2.7 ± 0.7 ab	4.1 ± 0.5 b	3.8 ± 0.2 b
	Submucosa (mg/cm)	1.8 ± 0.3	2.5 ± 0.8	1.2 ± 0.2	0.9 ± 0.1
	Total Weight (mg/cm)	3.6 ± 0.3	5.2 ± 0.7	5.4 ± 0.6	4.7 ± 0.2
	% Mucosa	50.6 ± 5.1 a	53.1 ± 9.7 a	76.9 ± 4.0 b	79.8 ± 2.2 b
ILEUM	Mucosa (mg/cm)	1.5 ± 0.3	2.1 ± 0.2	2.8 ± 0.7	1.9 ± 0.3
	Submucosa (mg/cm)	1.9 ± 0.3	1.2 ± 0.2	1.6 ± 0.5	1.5 ± 0.3
	Total Weight (mg/cm)	3.5 ± 0.3	3.3 ± 0.2	4.4 ± 0.6	3.4 ± 0.2
	% Mucosa	44.8 ± 5.8	64.8 ± 5.0	61.8 ± 9.0	55.9 ± 8.0

Values are expressed mean ± sem.
a, b : values with different letters are significantly different $p < 0.05$ by ANOVA
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The suckling animals were sacrificed on day 19-21. There were 8 suckling rats per treatment group.

Table 37. Intestinal characteristics of weanling rats of dams injected through pregnancy and lactation with GLP-2, DEX, or GLP-2 + DEX

		CONTROL	GLP-2	DEX	GLP-2+DEX
JEJUNUM	Mucosa (mg/cm)	8.7 ± 2.0	8.9 ± 1.3	8.8 ± 0.8	8.5 ± 1.1
	Submucosa (mg/cm)	1.0 ± 0.1	1.2 ± 0.16	0.9 ± 0.1	1.0 ± 0.2
	Total Weight (mg/cm)	10.0 ± 2.0	10.1 ± 1.2	9.7 ± 0.8	9.5 ± 0.9
	% Mucosa	87.9 ± 2.5	89.7 ± 2.3	90.0 ± 1.9	87.7 ± 2.6
ILEUM	Mucosa (mg/cm)	7.9 ± 1.2	10.5 ± 2.1	8.8 ± 0.7	8.3 ± 0.6
	Submucosa (mg/cm)	0.7 ± 0.1	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
	Total Weight (mg/cm)	8.6 ± 1.1	11.5 ± 2.0	9.5 ± 0.6	9.2 ± 0.6
	% Mucosa	89.8 ± 2.6	89.8 ± 2.8	91.3 ± 1.8	89.9 ± 1.5

Values are expressed mean ± sem.

None of these differences was statistically significant tested by ANOVA

The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The weanling animals were sacrificed 28 days after the weaning. There were 8 rats per treatment group.

Table 38. The effect of treatment of pregnant and lactating rat dams with GLP2, DEX, or GLP2 + DEX on jejunal morphology of suckling rats

Jejunum (µm)	**CONTROL**			**GLP-2**			**DEX**			**GLP-2+DEX**		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous Height	269.6	±	34.7	353.6	±	92.5	278.6	±	39.9	318.8	±	20.4
Villous Width (base)	72.0	±	2.0	83.4	±	16.1	73.5	±	11.5	82.3	±	11.6
Villous Width (half height)	63.7	±	2.7	70.2	±	15.8	65.5	±	7.0	61.0	±	7.9
Crypt Depth	47.8	±	3.8	70.6	±	13.2	55.6	±	11.5	58.1	±	4.0
Distance /5 villi	509.1	±	38.6	674.7	±	85.8	540.4	±	28.7	516.8	±	28.5
Distance /5 cells	29.8	±	0.5**a**	45.8	±	6.3**b**	31.6	±	1.4**a**	33.7	±	2.4**a**

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA.
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The suckling rats were sacrificed on day 19-21. In each group were 8 rats.

Table 39. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2 + DEX on ileal morphology of suckling rats

Ileum (µm)	CONTROL			GLP-2			DEX			GLP-2+DEX		
Suckling	Mean		Sem	Mean		Sem	Mean		Sem	Mean		Sem
Villous Height	218.7	±	9.7**a**	274.7	±	14.7**b**	184.9	±	19.0**a**	190.8	±	15.2**a**
Villous Width (base)	57.1	±	2.7	92.4	±	19.2	69.0	±	12.1	53.2	±	4.8
Villous Width (half height)	57.5	±	1.9	77.5	±	12.4	59.4	±	5.5	47.0	±	5.6
Crypt Depth	32.7	±	3.2	60.2	±	12.2	35.6	±	5.5	32.4	±	2.6
Distance /5 villi	425.2	±	15.7	602.2	±	87.5	517.1	±	62.0	424.7	±	17.0
Distance /5 cells	27.3	±	2.0	30.6	±	2.2	28.9	±	1.9	28.3	±	0.4

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA.
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The suckling rats were sacrificed on day 19-21. In each group were 8 suckling rats.

Table 40. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2 + DEX on jejunal morphology of weanling rats

Jejunum (µm)	**CONTROL**			**GLP-2**			**DEX**			**GLP-2+DEX**		
Weanling	Mean		Sem	Mean		Sem	Mean		sem	Mean		Sem
Villous Height	346.8	±	54.0**a**	822.5	±	36.4**b**	866.6	±	67.0**b**	897.9	±	53.5**b**
Villous Width (base)	131.1	±	8.0**a**	240.9	±	16.0**b**	226.2	±	22.2**b**	251.2	±	20.9**b**
Villous Width (half height)	108.0	±	12.4**a**	174.6	±	10.5**b**	174.0	±	11.7**b**	156.4	±	13.4**b**
Crypt Depth	80.7	±	14.5**a**	177.9	±	1.9**b**	154.9	±	15.4**b**	139.7	±	4.2**b**
Distance /5 villi	811.6	±	115.0	1218.9	±	9.1	1027.0	±	5.7	1090.9	±	68.2
Distance /5 cells	54.0	±	15.8	60.7	±	8.8	56.2	±	2.2	57.6	±	5.5

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA.

The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The weanlings were sacrificed 28 days after weanling and there were 8 rats in each group

Table 41. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2 + DEX on ileal morphology of weanling rats

Ileum (µm)	**CONTROL**			**GLP-2**			**DEX**			**GLP-2+DEX**		
Weanling	Mean		Sem	Mean		Sem	Mean		sem	Mean		Sem
Villous Height	287.8	±	43.5	310.4	±	77.0	263.3	±	18.5	239.1	±	16.4
Villous Width (base)	116.5	±	14.3	116.0	±	33.0	105.3	±	7.3	112.9	±	4.5
Villous Width (half height)	112.9	±	20.5	104.6	±	37.0	94.9	±	1.3	95.1	±	4.8
Crypt Depth	88.2	±	12.1	86.6	±	29.1	87.7	±	7.1	103.7	±	13.2
Distance /5 villi	893.3	±	170.1	838.1	±	153.3	670.2	±	31.6	703.4	±	44.8
Distance /5 cells	42.0	±	7.3	30.9	±	4.0	26.1	±	1.1	27.0	±	2.2

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA.
The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The weanlings were sacrificed 28 days after weanling and there were 8 rats in each group

Table 42. Effect of treatment of dams during pregnancy and lactation on jejunal and ileal uptake (Jd) of lipids in suckling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	28.5±4.4 a	32.6±5.4 a	10.5±2.1 b	10.8±2.2 b
	16:0	2.1±0.2	2.6±0.4	1.8±0.4	2.6±0.5
	18:0	2.5±0.4 ab	3.7±0.7 a	1.8±0.1 b	2.3±0.4ab
	18:1	2.0±0.2	2.2±0.5	1.4±0.2	2.4±0.4
	18:2	1.8±0.3 ab	2.6±0.5 a	1.2±0.1b	1.7±0.2 ab
	18:3	1.4±0.2	1.9±0.3	1.6±0.4	2.1±0.2
	Chol	1.2±0.2 b	2.2±0.5a	1.3±0.2 b	0.9±0.2 b
Ileum	12:0	40.2±6.7 a	34.6±3.5 a	4.6±1.0 b	22.1±3.0 ab
	16:0	3.3±0.4	5.8±0.9	1.6±3.3	2.0±0.4
	18:0	3.5±0.4 ab	4.5±0.7 a	1.8±0.5 b	2.1±0.4 b
	18:1	4.3±0.5 a	1.2±0.2 b	1.4±0.3 b	3.0±0.3 ab
	18:2	3.8±0.5 a	2.2±0.4 b	1.4±0.2 b	2.1±0.4 b
	18:3	3.1±0.4	3.1±0.6	2.0±0.3	2.1±0.3
	Chol	2.1±0.3 a	1.8±0.3 a	1.2±0.2 ab	[illegible]5±0.1 b

Values are mean ± sem a, b: values with different letters are significantly different p<0.05 by ANOVA. Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The suckling animals were sacrificed on day 19-21 and there were 8 suckling rats in each group.

Table 43. Effect of treatment of dams during pregnancy and lactation on jejunal and ileal uptake (Jm) of lipids in suckling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	62.7±10.4 a	53.7±9.4 a	18.2±4.0 b	28.9±4.2 b
	16:0	4.7±0.5	4.5±0.6	2.4±0.5	3.2±0.7
	18:0	5.0±0.7 a	5.5±0.8 a	2.4±0.2 b	2.9±0.5 b
	18:1	3.7±0.5	3.55±0.7	1.7±0.3	3.0±0.6
	18:2	3.5±0.6	4.5±1.9	1.6±0.2	1.2±0.3
	18:3	2.0±0.3	2.7±0.4	2.0±0.5	2.6±0.3
	Chol	1.7±0.3 ab	3.3±0.8 a	1.7±0.3 ab	1.1±0.2 b
Ileum	12:0	98.9±16.4 a	58.2±5.7 b	24.7±10.2 b	46.4±5.5 b
	16:0	5.6±0.9 ab	8.2±1.2 a	2.5±0.3 b	4.2±0.8 b
	18:0	10.1±1.6 a	6.8±0.8 ab	3.0±1.4 b	4.3±0.6 b
	18:1	12.8±2.4 a	2.5±0.5 b	4.4±1.9 b	5.6±0.6 b
	18:2	9.8±1.8 a	3.8±0.5 b	2.1±0.4 b	4.9±1.3 b
	18:3	4.7±0.6	5.2±0.8	3.4±0.4	4.1±0.5
	Chol	2.8±0.5 a	2.8±0.5 a	2.0±0.4 ab	1.0±0.1 b

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The suckling animals were sacrificed on day 19-21 and there were 8 suckling rats in each group.

Table 44. Effect of treatment of dams during pregnancy and lactation on jejunal and ileal uptake (Jd) of lipids in weanling rats

Jejunum	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
	12:0	15.5±1.3 a	14.9±1.5 a	11.6±1.7 a	10.6±1.7 b
	16:0	1.5±0.1	1.5±0.1	1.3±0.1	1.5 ±0.2
	18:0	1.3±0.1	1.6±0.1	1.5±0.2	1.0±0.1
	18:1	1.4±0.1	1.5±0.1	1.0±0.1	1.2±0.1
	18:2	1.0±0.1	1.5±0.1	1.2±0.2	1.2±0.1
	18:3	1.1±0.1	1.3±0.1	1.2±0.1	1.0±0.1
	Chol	0.7±0.1 ab	0.9±0.1 a	1.0±0.1 a	0.6±0.1 b
Ileum	12:0	18.8±1.4 a	12.5±0.9 b	12.8±1.5 b	13.5±1.2 b
	16:0	1.1±0.1 a	1.3±0.1 a	1.1±0.1 ab	0.8±0.1 b
	18:0	1.1±0.1 ab	1.4±0.1 a	1.0±0.1 b	1.0±0.1 a
	18:1	1.3±0.1 a	1.0±0.8 b	0.8±0.1 b	0.7±0.1 b
	18:2	1.0±0.1 ab	1.2±0.1 a	0.7±0.1 b	0.9±0.1 a
	18:3	1.0±0.1 a	0.8±0.1 b	0.8±0.1 b	0.8±0.1 b
	Chol	0.9±0.1 b	0.8±0.1 b	1.0±0.1 a	0.6±0.1 b

Values are mean ± sem a, b: values with different letters are significantly different p<0.05 by ANOVA. Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The weanling animals were sacrificed 28 days after the weaning and there were 8 rats in each group.

Table 45. Effect of treatment of dams during pregnancy and lactation on jejunal and ileal uptake (Jm) of lipids in weanling rats

	FA	CONTROL mean±sem	GLP-2 mean±sem	DEX mean±sem	GLP-2 + DEX mean±sem
Jejunum	12:0	17.8±1.5	17.3±1.7	12.9±1.9	12.3±2.1
	16:0	1.6±0.1	1.8±0.1	1.4±0.2	1.6 ±0.3
	18:0	1.5±0.8 ab	1.9±0.2 a	1.9±0.2 a	1.0±0.1 b
	18:1	1.5±0.2 ab	1.7±0.1 a	1.2±0.1 b	1.4±0.1 ab
	18:2	1.2±0.1	1.6±0.1	1.4±0.2	1.3±0.2
	18:3	1.3±0.2	1.5±0.1	1.4±0.2	1.1±0.1
	Chol	0.9±0.1 ab	1.1±0.2 a	1.1±0.1 a	0.7±0.1 b
Ileum	12:0	21.0±1.6 a	14.0±0.1 b	13.8±1.8 b	15.0±1.3 b
	16:0	1.2±0.1	1.4±0.1	1.3±0.1	0.9±0.1
	18:0	1.3±0.1 ab	1.6±0.2 a	1.0±0.1 b	1.1±0.1 b
	18:1	1.3±0.1 a	1.1±0.1 ab	0.9±0.2 b	0.8±0.1 b
	18:2	0.9±0.1 b	1.4±0.2 a	0.8±0.1 b	1.1±0.1 b
	18:3	1.2±0.1 a	0.8±0.1 b	0.8±0.1 b	1.0±0.1 ab
	Chol	1.0±0.1	1.0±0.2	1.1±0.1	0.7±0.1

Values are mean ± sem a, b: values with different letters are significantly different $p<0.05$ by ANOVA. Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The weanling animals were sacrificed 28 days after the weaning and there were 8 rats in each group.

Table 46. Comparison of lipid uptake (Jd) into the jejunum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	28.5±4.4	15.5±1.3 #	32.6±5.4	14.9±1.5 #	10.5±2.1	11.6±1.7	10.7±2.2	10.6±1.7
16:0	2.1±0.2	1.5±0.1 #	2.6±0.4	1.5±0.1 #	1.8±0.4	1.3±0.1	2.6±0.5	1.5±0.2
18:0	2.5±0.4	1.3±0.1 #	3.7±0.7	1.6±0.1 #	1.8±0.13	1.5±0.2	2.3±0.4	1.0±0.1 #
18:1	2.0±0.2	1.3±0.1 #	2.2±0.5	1.5±0.1	1.4±0.2	1.0±0.1	2.4±0.4	1.2±0.1 #
18:2	1.8±0.3	1.0±0.1 #	2.6±0.5	1.5±0.1	1.2±0.1	1.2±0.2	1.7±0.2	1.2±0.1
18:3	1.4±0.2	1.1±0.1	1.9±0.3	1.3±0.1	1.6±0.4	1.2±0.1	2.1±0.2	1.0±0.1 #
Chol	1.2±0.2	0.7±0.1 #	2.2±0.5	0.9±0.1 #	1.3±0.2	1.0±0.1	0.8±0.2	0.6±0.1

Values are expressed as mean ± sem.
#: significantly different by t-test, $p<0.05$ sucklings versus weanlings
Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 47. Comparison of lipid uptake (Jm) into the jejunum of suckling vs weanling rats

FA	**CONTROL**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	62.6±10.4	17.8±1.7#	53.7±9.4	17.30±1.7#	18.2±4.0	12.9±1.9	28.9±4.2	12.3±2.1#
16:0	4.7±0.5	1.6±0.1#	4.5±0.6	1.8±0.1#	2.4±0.5	1.4±0.2	3.2±0.7	1.6±0.2#
18:0	5.0±0.7	1.5±0.2#	5.5±0.8	1.9±0.2#	2.4±0.2	1.9±0.2	2.9±0.6	1.0±0.1#
18:1	3.7±0.5	1.5±0.2#	3.6±0.7	1.7±0.1#	1.7±0.3	1.2±0.01	3.1±0.6	1.4±0.1#
18:2	3.5±0.6	1.2±0.1#	4.5±1.9	1.6±0.1	1.6±0.2	1.4±0.2	2.0±0.3	1.3±0.2#
18:3	2.0±0.3	1.3±0.2#	2.7±0.4	1.5±0.1#	2.0±0.5	1.4±0.2	2.6±0.3	1.1±0.1#
Chol	1.7±0.3	0.8±0.01#	3.3±0.8	1.0±0.2#	1.7±0.3	1.1±0.1	1.1±0.2	0.6±0.1#

Values are expressed as mean ± sem.
#: significantly different by t-test, $p<0.05$ sucklings versus weanlings
Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 48. Comparison of lipid uptake (Jd) into the ileum of suckling vs weanling rats

FA	**CONTROL**		**GLP-2**		**DEX**		**GLP-2 + DEX**	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	40.2±6.6	18.7±1.4 #	34.6±3.5	12.5±0.9 #	4.6±1.0	12.8±1.5 #	22.1±3.0	13.5±1.2 #
16:0	3.3±0.4	1.1±0.1 #	5.8±0.9	1.3±0.1 #	1.6±3.3	1.1±0.1	2.0±0.4	0.8±0.1 #
18:0	3.5±0.4	1.1±0.1 #	4.5±0.7	1.4±0.1 #	1.8±0.5	1.0±0.1	2.1±0.4	1.0±0.1 #
18:1	4.3±0.5	1.3±0.1 #	1.2±0.2	1.0±0.1	1.4±0.3	0.8±0.1	3.0±0.3	0.7±0.1 #
18:2	3.8±0.5	1.0±0.1 #	2.2±0.4	1.2±0.1 #	1.4±0.2	0.7±0.1 #	2.1±0.4	0.9±0.1 #
18:3	3.1±0.4	1.1±0.1 #	3.1±0.6	0.8±0.1 #	2.0±0.3	0.7±0.1 #	2.1±0.3	0.8±0.1 #
Chol	2.1±0.3	0.8±0.1 #	1.8±0.3	0.8±0.1 #	1.2±0.2	1.0±0.1	0.5±0.1	0.6±0.1

Values are expressed as mean ± sem.
#: significantly different by t-test, $p<0.05$ sucklings versus weanlings
Jd : uptake rate calculated base on total tissue weight (nmol 100 mg^{-1} tissue weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 49. Comparison of lipid uptake (Jm) into the ileum of suckling vs weanling rats

FA	CONTROL		GLP-2		DEX		GLP-2 + DEX	
	suckling vs weanling		suckling vs weanling		suckling vs weanling		suckling vs weanling	
12:0	99.9±16.4	21.0±1.6#	58.2±5.7	14.4±1.0#	24.7±10.2	13.8±1.8	46.4±5.5	15.0±1.3#
16:0	5.6±0.9	1.2±0.1#	8.2±1.2	1.4±0.1#	2.5±0.3	1.3±0.2#	4.1±0.8	0.9±0.1#
18:0	10.1±1.6	1.3±0.1#	6.8±0.8	1.6±0.2#	3.0±1.4	1.0±0.1	4.3±0.7	1.1±0.1#
18:1	12.8±2.4	1.3±0.1#	2.5±0.5	1.1±0.1#	4.4±1.9	0.9±0.2	5.6±0.6	0.8±0.1#
18:2	9.7±1.8	1.0±0.1#	3.8±0.6	1.4±0.2#	2.0±0.4	0.8±0.1#	4.9±1.3	1.1±0.1#
18:3	4.7±0.6	1.2±0.1#	5.2±0.8	0.8±0.1#	3.4±0.4	0.8±0.1#	4.1±0.6	1.0±0.1#
Chol	2.8±0.5	1.0±0.1#	2.8±0.5	1.0±0.2#	2.0±0.4	1.1±0.1#	1.0±0.1	0.7±0.1#

Values are expressed as mean ± sem.
#: significantly different by t-test, $p<0.05$ sucklings versus weanlings
Jm : uptake rate calculated base on mucosa weight (nmol 100 mg^{-1} mucosa weight min^{-1}). The treatments include GLP-2 (0.1 µg / g twice a day), DEX (0.128 µg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 50. Comparison of the abundance of I-FABP and L-FABP in suckling versus weanling rats in jejunum and ileum of the Control group

		Suckling			Weanling		
I - FABP	CONTROL jejunum	61.9	±	5.2	58.3	±	2.0
	CONTROL ileum	78.3	±	3.6	72.2	±	4.7
L - FABP	CONTROL Jejunum	69.6	±	2.6	62.3	±	2.5
	CONTROL ileum	64.9	±	2.2	59.0	±	3.4

Values are ratio of area stained with antibody versus total tissue area and are expressed as mean ± sem

None of these differences was statistically significant when tested by t-test

The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

Table 51. I-FABP and L-FABP in the ileum of offspring whose mothers received treatment during pregnancy and lactation

	CONTROL			GLP-2			DEX			GLP-2+DEX		
I-FABP												
Suckling	78.3	±	3.6	71.4	±	4.7	76.7	±	2.6	66.0	±	3.3
Weanling	72.2	±	4.7**b**	65.1	±	3.0**b**	67.8	±	3.7**b**	58.0	±	0.9**a**
L-FABP												
Suckling	64.9	±	2.2	67.8	±	5.7	58.5	±	3.2	60.9	±	5.5
Weanling	59.0	±	3.4	60.7	±	4.0	62.0	±	2.3	55.3	±	3.0

Values are pixels per area and are expressed as mean ± sem. a, b: values with different letters are significantly different $p<0.05$ by ANOVA. The treatments include GLP-2 (0.1 μg / g twice a day), DEX (0.128 μg / g once a day), and GLP-2 + DEX at those doses, given during pregnancy and lactation. The sucklings were sacrificed on day 19-21 and the weanlings were sacrificed 28 days after the weaning. In each group were 8 rats.

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CHAPTER 6

GENERAL DISCUSION

6.1 GLUGAGON-LIKE PEPTIDE-2

Intestinal failure is an uncommon but serious condition (Fishbein et al. 2002, Ward et al. 2003). The use of GLP-2 has been attempted with some success in an effort to accelerate the intestinal adaptive process in adults with the short bowel syndrome, and thereby to enhance the ability of the intestine to absorb nutrients (Jeppesen et al. 2001). This human experience supports the evidence from animal studies of GLP-2 having a trophic and pro-absorptive effect on the intestinal absorption of sugars (Cheeseman et al. 1996, 1997, 1998) and triolein (Brubaker et al. 1997). The mRNA for the GLP-2R is even higher in neonatal than in adult intestine (Burrin et al. 2000, Lovshin et al. 2000). Therefore, we had anticipated that GLP-2 given to suckling rats would have a trophic effect on the intestine, possibly as a result of its pro-proliferative and anti-apoptotic effects (Burrin et al. 2000, Drucker 1996, 2003), as well as its pro-absorptive effect (Cheeseman et al. 1996, 1997, 1998, Brubaker et al. 1997). If this were the case, then GLP-2 might possibly be useful for the treatment of infants with the short bowel syndrome. To our considerable surprise, a 10 day course of twice daily GLP-2 given to suckling rats in doses that were effective in adults had no effect on the intestinal uptake of fatty acids or cholesterol (Paper 1). GLP-2 given to sucklings also had no trophic effect. This important series of findings suggests that GLP-2 is not capable of stimulating intestinal growth or accelerating lipid uptake in newborns, and would likely be of limited clinical benefit in this setting.

Because some pregnant mothers with intestinal failure may be treated with GLP-2, it is important to know if GLP-2 given to the mother would affect the form or function of the offspring's intestine. It is not know if GLP-2 crosses the placenta or goes into mother's milk. We choose to undertake these studies before having this information, because we recognized the possibility that GLP-2 given to the pregnant or lactating mother might stimulate other peptides which were themselves trophic to the morphology of the intestine and stimulatory for its function, and by crossing the placenta or into milk, would be biologically active. Candidate peptides include hepatocyte growth factor, fibroblast growth factor, platelet-derived growth factor, and granulocyte growth factor (Calhoun et al. 1999, 2000, 2001, Sukhotnik et al. 2003). Because GLP-2 given directly to the suckling rats had no effect on intestinal transport and no trophic effect on morphology (Paper 1), we were surprised to observe that GLP-2 given to the pregnant and lactating rat dams resulted in ileal lipid malabsorption in the sucklings (Paper 3). This effect is likely due to a process which occurred during pregnancy rather that during lactation, since injecting the lactating dams with GLP-2 had no effect on lipid uptake in the sucklings (Paper 2). Thus, there is a consideration that pregnant mothers should not be given GLP-2 because of the possibility of lipid malabsorption in their babies.

There is a further concern for the potential adverse effect of giving GLP-2 to pregnant or lactating dams: the effect of the GLP-2 injections persisted for at least four weeks after their administration was stopped (Papers 2 and 3). For example, when the dams were given GLP-2 in pregnancy and lactation, the ileal malabsorption of lipids persisted in weanling (Paper 3). When GLP-2 was given to the dams just during the period of lactation, they developed jejunal and ileal malabsorption of 12:0, 16:0, and 18:1, and hyperabsorption of 18:2 and cholesterol into the ileum of the weanlings (Paper 2). Thus, there is a concern that giving GLP-2 during pregnancy and lactation may be harmful to the normal lipid absorption function of the suckling offspring: even when there was no deleterious effect in sucklings, an alteration may occur a month later (Paper 2), or the malabsorption of lipids observed in suckling persists into the weanling period (Paper 3).

6.2 GLUCOCORTICOISTEROIDS

Glucocorticosteroids promote the intestinal absorption of nutrients such as sugars and lipids in adult animals, even though they may result in some atrophy of the intestine (Park et al. 1994, Kritsch et al. 2000). Recognizing the potential clinical need for an agent that could be used to enhance nutrient absorption in the newborn, and appreciating that a major source of calories arise from the lipids which are present in abundance in mother's milk (Casey and Hambidge 1983, Boersma et al. 1991), we wished to determine the influence of GC on lipid uptake into the intestine of suckling rats.

Examining the possibility for continuation of this effect into the weanling period was highlighted by the observation of the persistent or late effect of GLP-2 on the intestinal uptake of lipids (Papers 2 and 3). In addition, previous authors have identified the concept of the "critical period phenomenon" (Karasov et al. 1985, Pacha et al. 2000). It is recognized that even simple isocaloric changes in the lipid composition of the pregnant and /or lactating dam's diet will modify the intestinal transport function in the offspring (Jarocka-Cyrta et al. 1998, Nilza et al. 1999). The intestine of suckling animals responds to the injection of DEX by enhancing the jejunal and ileal uptake of some long-chain fatty acids (Paper 1). This enhanced uptake of fatty acids and cholesterol was also seen in the jejunum of suckling rats whose mothers had been given DEX during lactation (Paper 2). In marked contrast, giving DEX during pregnancy and lactation resulted in malabsoption of lipids in the ileum (Paper 3). This stresses the point that the effect of DEX when given to adults or to suckling rats, or even to lactating dams, has an opposite effect when given during pregnancy. Therefore, the safety of a treatment during pregnancy cannot be inferred from findings at a time other than pregnancy.

Furthermore, just as the effect of DEX on the stimulation of lipid uptake into the intestine of sucklings persists for at least four weeks after the last injection of GC (Paper 1), so also the malabsorption of lipids which occurs when DEX is given to pregnant and lactating rats persist for a month after the last injection (Paper 3). We did not establish the mechanism of this persistent effect of DEX, but we speculate that the GC acting through its nuclear receptor increased the abundance of the mRNA for one or more factors which altered the ontogeny of the intestine for the uptake of lipids.

It is not clear whether this persistent effect of DEX on intestinal lipid uptake was necessarily deleterious. The uptake of some lipids remained elevated in weanlings injected previously with DEX during the suckling period (Paper 1). On the other hand, giving DEX during pregnancy and lactation resulted in a persistent malabsorption of lipids (Paper 3). Furthermore, lipid uptake normally falls between the suckling and weanling periods (Frost et al. 1983, Flores et al. 1989), concurrent with the normal switch from a high fat milk diet to the low fat high carbohydrate diet consumed after weaning. However, DEX given during pregnancy and lactation prevents this expected decline in lipid uptake into the jejunum of weanlings as compared with sucklings (Paper 3). Some mothers may need to use GC during pregnancy and lactation for a medical condition, such as asthma or inflammatory bowel disease. GC pass across the placenta and into milk; the early enhancing effects on lipid uptake into the jejunum of sucklings (Paper 2), or the early and persistent malabsorption of lipids in sucklings and weanlings of dams given DEX during pregnancy and lactation (Paper 3), raises the cautionary note for the need to monitor the intestinal function in children whose mothers consumed GC during the perinatal period.

6.3 GLUGAGON-LIKE PEPTIDE-2 PLUS GLUCOCORTICOSTEROIDS

Either GLP-2 or GC are capable of stimulating nutrient uptake in adults (Cheeseman et al. 1996, 1997, 1998, Brubaker et al. 1997, Jeppesen et al. 2001, Batt and Scott 1982, Crake et al. 1984, Thiesen et al. 2002, 2003), thus, we speculated that the combination of GLP-2 plus DEX would enhance lipid uptake even more than either agent alone. However, there was no evidence of an additive or synergistic effect, although adding GLP-2 to DEX given to the suckling animals increased the lipid uptake seen in ileum of weanling offspring (Paper 1). In addition, the ileal malabsorption of lipids seen in sucklings and weanlings whose pregnant and lactating dams were treated with DEX was not prevented by adding GLP-2 (Paper 1). Furthermore, when the lactating dams were treated, adding GLP-2 to DEX resulted in increases in the uptake of some lipids and decreases in the uptake of others, which was not observed with DEX alone (Paper 2). Adding GLP-2 to DEX did not accentuate any benefits or reduce any deleterious effects of DEX given alone.

GLP-2 and GC have opposite effects on the morphology of the intestine: GLP-2 stimulates proliferation and inhibits apoptosis (Burrin et al. 2000, Drucker 1996, 2003), whereas GC inhibits proliferation and stimulates apoptosis (Batt and Peters 1976, Park et al. 1994, Kritsch et al. 2000). We speculated that adding GLP-2 to DEX would prevent any possible intestinal atrophy that would result from the DEX alone. Indeed, when lactating dams were given GLP-2 plus DEX, the loss of body weight and the loss of mucosal weight of the suckling intestine, observed with DEX alone, was prevented (Paper 2). In addition, when DEX was given to sucklings, there was a loss of body weight in the weanlings, and this was also prevented by giving GLP-2 plus DEX (Paper 1).

We expected that the effects on lipid uptake generated by treatments with GLP-2, DEX, or GLP-2+DEX will persist in later life due to exposure of the intestine to these stimuli during 'critical period programming period', and there were found long-term effect in all experiments (Paper 1, 2, 3). For this reasons, we cannot recommend the use of GLP-2, DEX or GLP-2 + DEX to enhance lipid uptake during the neonatal period. Furthermore, because it is not known what might be the nutritional or the metabolic effect of altered lipid uptake described in these studies, and the possibility that these changes last long after the treatment is stopped, we recommend caution in the use of either GLP-2 or DEX in pregnancy and lactating mothers.

6.4 MECHANISMS

Lipid absorption is a complex process (Schultess et al. 2000). Once lipids in the intestinal lumen are digested and solubilized in bile salt and phospholipids micelles, they diffuse across the intestinal UWL, and permeate the BBM either through the lipophilic portion of the membrane, or their uptake is assisted by lipid binding proteins in the BBM or enterocyte cytosol. The effective resistance of the UWL is determinate by the diffusion coefficient of the probe, and by the thickness and surface area of the UWL (Thomson et al. 2003). The resistance of the UWL changes in health and disease. For example, UWL resistance falls after external abdominal radiation or with aging, and increases in diabetes (Thomson et al. 1984, Hollander et al. 1984, Hotke et al. 1985). It is unknown how the UWL resistance changes under these conditions. The diffusion coefficient of the probe in the UWL could be modified by the viscosity of the UWL, which in turn may be influenced by the quality of the intestinal mucus. Although it is known that GC increases the number of goblet cells per villi and their secretion of mucus, there is no information about how GLP-2 may affect the intestinal mucus (Castagliuolo et al. 1996, Gordon et al. 2000). Using the rate of uptake of 12:0 as a measure of UWL resistance (Westergaard and Dietschy 1974), the UWL resistance fell in the ileum of weanlings previously directly injected with GLP-2 + DEX during the suckling period (Paper 1), and UWL resistance rose in the jejunum and ileum of weanlings whose mothers had been given GLP-2 (Paper 2). As well, UWL resistance rose in the ileum of sucklings and weanlings whose pregnant and lactating dams were given GLP-2, DEX, GLP-2 + DEX (Paper 3). However, in each of these examples, the alteration in the uptake of the long chain fatty acids and cholesterol was appropriate for the variations in UWL resistance (decreased uptake with increased UWL resistance, and vice versa).

The lipophilic properties of the BBM influence lipid uptake, as also does the fluidity of the BBM. For example, for a fall in lipid uptake, it would be expected that the BBM would be less lipophilic and that the BBM fluidity would decrease (Meddings

1988). It is recognized that lipid uptake falls between the suckling and weanling period (Frost et al. 1983, Flores et al. 1989), and we also observed this age-dependent decline (Papers 1, 2, 3). During this same interval, others have reported alterations in the BBM content of lipids, as well as a decline in the fluidity of the BBM (Schwarz et al. 1985, Hubner et al. 1988, Keelan 1997). We did not repeat these measurements of BBM fluidity or lipid content in the current studies. We speculate that the alterations in lipid uptake in response to GLP-2, DEX, or GLP-2 + DEX may have been due partially to altered BBM permeability, as well as to variations in the UWL resistance.

There are many lipid binding proteins in the enterocyte cytosol and BBM (Besnard and Niot 2000). It was speculated that some of these may be involved in lipid uptake. For example, using an antibody to the fatty acid binding protein in BBM reduced the lipid uptake (Stremmel 1988, Schoeller et al. 1995). Feeding animals a high fat diet increases the intestinal lipid uptake and enhances the abundance of L-FABP (Poirier et al. 1997, Besnard and Niot 2000). The exposure of Caco-2 cells in culture to lipids increases both L-FABP and I-FABP in their cytosol (Dube et al. 2001). However, the alterations in lipid uptake in diabetes, aging, or in response to intestinal resection are not correlated with variations in enterocyte abundance of I-/L-FABP (Drozdowsky et al. 2001, Thiesen 2002, Woudstra 2002). Furthermore, in knockout mice lacking I-FABP, lipid uptake is normal under basal conditions (Agellon et al. 2002). In this series of studies, there was no change in I-/L-FABP when suckling rats were given DEX or GLP-2 + DEX, and yet lipid uptake increased (Paper 1). When pregnant and lactating dams were given GLP-2, DEX, or GLP-2 + DEX, the ileal malabsorption of lipids in sucklings and weanlings was not associated with alterations in the abundance of I-/L-FABP (Paper 3). Injecting lactating dams with GLP-2 or DEX did not result in alterations in the abundance of I-/L-FABP in the suckling intestine or in the weanling intestine. Finally, the suckling rats whose mothers were given GLP-2 + DEX showed an increase in lipid uptake in both the jejunum and the ileum, but I-/L-FABP increased in abundance only in the ileum (Paper 2). These proteins, I-/L-FABP, are present in the intestine of suckling and weanling rats, but their abundance either does not change, or changes in a minor way which does not explain any associated alteration in lipid uptake.

We suggest that these proteins do not play a role in the modification of lipid uptake in the perinatal period. Of course, it is possible that other lipid binding proteins, which we did not measure, may be responsible for the alterations in lipid uptake in response to GLP-2 or DEX, and in the fall in lipid uptake which occur between the suckling and weanling animals.

6.5 FUTURE STUDIES

GLP-2 and GC have opposite effects on the intestinal morphology. GLP-2 is a strong stimulus for proliferation and an inhibitor of apoptosis (Burrin et al. 2000, Drucker 1996, 2003), while GC inhibits proliferation and stimulates apoptosis (Batt and Peters 1976, Park et al. 1994, Kritsch et al. 2000). We considered that by adding GLP-2 to DEX, the DEX-induced atrophy would be prevented. GLP-2 prevented the loss of body weight and mucosal weight of the suckling intestine when administrated to nursing rats (Paper 2), and also the loss of body weight in the weanlings when DEX was given to sucklings (Paper 1). Thus, future studies regarding apoptosis and proliferation of the cells in the intestine might be performed.

There are multiple regulatory elements acting simultaneously to modulate the ontogeny of the intestine. In addition to the well known CDX1, CDX2 and PDX1 proteins, the hepatocyte nuclear factor as well as GATA-4 have been proven to play roles as transcription factors (Boudreau et al. 2002). Activation of the kinases systems such as MAPK, PKA, PI-3k and Raf may also contribute with mitogenic signals to the development of the intestine (Croxtall et al. 2000, Jasleen et al 2000, Yousta et al. 2002). Thus, all these signals might be investigated during ontogeny to determine their possible correlation with the effects of these treatments.

It is not know what signals are turned on by DEX to increase or decrease lipid uptake. Similarly, the signals responsible for the action of GLP-2 are unknown, and particularly in the setting of GLP-2 having an effect when given during pregnancy and/or lactation, it is unclear if this is a dual effect of GLP-2, or an indirect effect of some other peptide. Future studies must be undertaken using proteomic as well as genomic approaches to determine the signals that mediate the ontogeny of the intestine, and how this process is modified with GLP-2 and DEX.

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